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JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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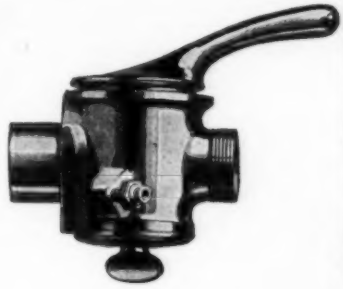

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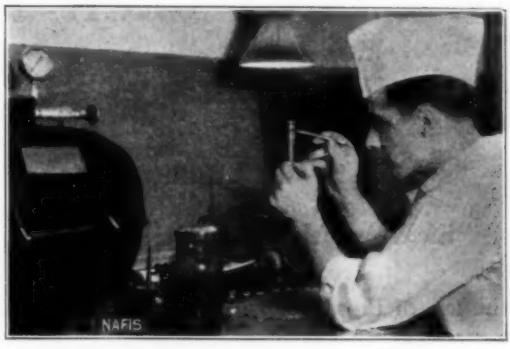

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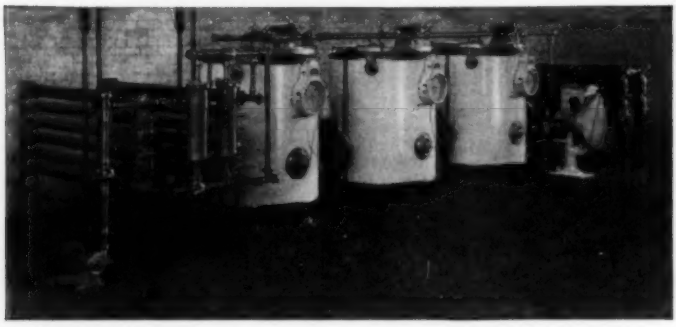

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CONTRIBUTION TO THE KNOWLEDGE OF THE TASTE OF MILK*

C. L. ROADHOUSE†

Department of Dairy Industry, University of California, Davis

AND

G. A. KOESTLER

Department of Chemistry, Swiss Dairy and Bacteriological Experiment Station, Liebefeld, Bern, Switzerland

Very little research has been reported dealing with the taste of milk at the time it is drawn from the cow, except as it is influenced by the use of certain feeds in the rations of dairy cows or by stable odors. In the scoring of milk as carried out in the United States, certain deviations from the normal taste of milk have been recognized, but complete knowledge of the causes of these variations has been lacking.

Milk, before it is delivered to the consumer, receives certain treatment, such as aeration and pasteurization, by means of which some minor defects in flavor may be more or less successfully removed, but there may be defects of taste which can not be removed by the usual methods of handling. It has been considered worth while, therefore, to study more closely the taste of raw, untreated milk and to discuss the individual variations in taste which are apparent in the milk from different cows.

PROCEDURE

The milk score card of the United States Department of Agriculture was used. All samples were heated to a temperature of 40° to 43°C. before they were tasted. Three experienced judges examined all samples of milk and when there was not complete

* Received for publication March 1, 1929.

† This study was conducted by the senior author while on sabbatical leave in Switzerland during 1927–1928. The authors are indebted to Mr. W. Loertscher of the Dairy Experiment Station at Liebefeld, Switzerland, for helpful collaboration in examining the flavor of the milk.

agreement, the results were averaged. In few instances was the variation greater than two-tenths of a point in the score.

From the preliminary trials it was evident that in spite of the most careful handling of the milk, including sanitation, cooling and withholding feed from the cows previous to milking, there was a difference in the taste of the milk from different cows and the individual milkings of the same cow. A careful study was made of the individual differences in taste and at the same time an effort was made to take a first step toward the analysis of the differences in taste.

The taste of milk, as this word is commonly used, is the sensation perceived when milk is taken into the mouth. The term "flavor," as used in this paper, is a combination of the sensations of taste in the mouth with those of smell produced through the medium of the inner nasal passages. The term "taste" is often used as synonymous with flavor, but it aids in precision to confine this term to those sensations which are perceived only in the mouth, and to use the term "flavor" to include also those sensations which are undetected if the milk is tasted while preventing the passage of air through the nasal passages. Odor or aroma, as applied to milk, describes the sensations observed when the milk is subjected to examination by the nasal passages alone. In tasting milk one must consider the impressions transmitted not only by the tongue, but also by the whole inner surface of the mouth. The correct taste impressions are more easily secured when the milk is taken in not too large amounts.

THE IDEAL MILK TASTE

At first thought, it appears somewhat daring to refer to an "ideal" milk taste. The taste trend of the individual consumer may vary somewhat from that of the milk-consuming public. Nevertheless, the ideas pertaining to the ideal milk taste are probably in closer agreement than are the ideas of the public concerning butter and cheese.

It is evident from the investigations which have thus far been made, that there is a type of milk which must be designated by every true connoisseur as one free from objection as far as taste is

concerned. After swallowing, an absolutely agreeable sensation must be left in the mouth. The milk is without odor under the usual conditions of examination, and the taste sensation which is given through the principal milk ingredients is even and full. No part of the taste as a whole predominates in such a way that the harmony is destroyed, but rather do the individual taste characteristics of the principal ingredients blend into one another. A further important requirement is that the milk must not give the impression of being thin or watery; the presence of the water should be masked by the combined taste sensation to such a degree that a flat, watery taste is not present.

ANALYSIS OF THE MILK TASTE

In the system of milk scoring used by other workers, a sharp distinction has not been made between the natural foundation taste and the secondary taste. There is no disadvantage recognized in this system, for the purely comparative judging of the taste. Just as soon, however, as one desires to approach the "analysis of the taste," then the necessity is felt for undertaking a certain basic classification. A first step in this direction was to consider what constitutes the foundation taste of the milk. It was decided that it should include all of the taste-giving ingredients which milk normally contains.

This "primary taste" of milk must be distinguished from the "secondary taste" or "flavor," which comprises defective body and everything of a foreign nature which the milk acquires, such as feed and stable flavors.

In attempting to analyze the taste of milk it became clear that the chemical composition of milk is most concerned with the foundation, or primary taste, of average milk; that feed and stable flavors are responsible for the secondary taste, and that the physical condition of the milk, which influences the body, serves to regulate the total impression by increasing or decreasing the pleasantness of the taste. The primary taste of milk cannot be successfully judged unless the conditions leading to secondary flavors are eliminated.

THE VARIATION IN THE TASTE OF MILK FROM NORMAL COWS

When tasting samples of milk collected from 4 dairy herds in Switzerland, the writers observed considerable variation in the taste of milk from individual cows. Among 82 cows from which

TABLE 1

Variations in taste and body of milk from apparently normal cows

NAME OF COW	SAMPLE NUMBER	FLAVOR SCORE	JUDGES COMMENTS	LENGTH OF TIME COW HAD BEEN MILKING
Flora	9	13.1	Slight salty, cowy	10 months, 3 days
Gritli	5	13.8	Very good taste	6 months, 24 days
Natter	1	13.8	Fine taste	9 months, 16 days
Nigeli	10	13.5	Aftertaste	4 months, 6 days
Lou	18	13.7	Slight watery	4 months, 19 days
Lusti	1	13.2	Chalky, uneven, lacks taste	10 months, 21 days
Bernina	5	13.3	Watery	1 month, 12 days
Rosette	7	10.8	Bad taste and odor, musty	16 days
Wanda	11	13.2	Slight salty	7 months, 18 days
Pupilla	14	13.0	High acid, uneven taste	3 years, 1 month, 21 days
Falk	8	13.4	Very slightly rancid	8 months, 21 days
Primula	1	13.4	Flat taste	5 months, 8 days
Rütli	17	13.7	Superb taste, thin body	
Pia	23	13.0	Acid-like taste	9 days
Spiri	4	13.1	Astringent, off taste	2 months, 1 day
Vion	9	13.0	Thin body, not pleasing	2 months, 22 days
Schägg	7	12.8	Sharp taste, salty	3 months, 9 days
Gemse	8	12.8	Thin, watery and bitter	9 months, 28 days
Blösch	6	12.8	Sharp, slightly bitter and sweet	
Türk	4	11.5	Slightly rancid odor, rancid taste	
Runde	2	12.6	Strong salty, aftertaste	
Spiess	6	12.6	Disagreeable odor and taste	
Tiger	1	0	Strong rancid odor and taste, bitter	12 months

samples were taken, there were 8 cows which produced milk with an especially fine taste; 35, with a good taste; 11, with a slightly salty taste; 2, distinctly salty; 4, slightly rancid; 5, distinctly rancid; and 4, with a bad taste.

Gamble and Kelly (1) have reported a wide variation in the taste and odor of the milk from individual normal cows receiving the same feeds, and one of us (2) has observed a similar variation in taste in milk from cows examined in California.

In table 1 are given the scores for certain cows' milk showing variation in taste. In each instance feed was not given to the cows during the five-hour period previous to milking.

The results show marked differences in the taste between certain samples of milk.

THE CHEMICAL COMPOSITION OF MILK WITH A NORMAL, PLEASING TASTE

In order to determine if the different milk samples with exceptionally pleasing primary tastes would also show certain agreements in their chemical composition, milk samples were taken from three selected cows, the milks of which were especially agreeable in taste. This was shown in the milk from the cows, Felder, Müller and Lisi. The milk from the latter cow showed a slight after-flavor, which, according to our previous definition, would be classified as secondary taste.

Table 2 gives the analytical results. It is certainly not to be considered accidental that these three milk samples with excellent primary tastes should agree in lactose content to within a few hundredths per cent. An approximate agreement in chloride content is also shown.

Without endeavoring to obtain positive proof from these three examples, it can be assumed that the agreement of the chemical analyses with the judges' impressions of taste is close. It might also be noted that no especially close agreement was found in coarsely dispersed components such as fat, protein, and some ash constituents.

These results, therefore, furnish certain confirmation of the above statement, that the coarsely dispersed components exert an insignificant influence on the primary taste of milk.

TABLE 2

Analyses of milk with an especially good and even taste

	cow		
	Müller	Felder	Lisi
Physico-chemical analyses:			
Specific gravity at 15°C	1.02957	1.03408	1.03193
Acidity (calculated in per cent acid from Soxhlet-Henkel deg.)	0.13	0.14	0.12
Hydrogen ion concentration (pH)	6.70	6 68	6.63
Electrical conductivity (Mhos × 10^{-4})	41.79	39.37	44.09
Stalegmometer (J. Traube) S.T.:			
a. Drop count	38	37	37
b. Time (seconds)	36	36	42
Chlorine-lactose number	1.896	1.691	1.692
Chemical composition (per cent):			
Fat content (Röse-Gottl.)	3.65	3.79	4.96
Lactose hydrate	4.92	4.92	4.94
Total protein	2.639	2.591	3.599
Casein	1.85	2.14	2.83
Albumin, globulin	0.38	0.36	0.46
Citric acid	0.262	0.269	0.279
Ash	0.695	0.689	0.739
Total	12.166	12.559	14.317
Total dry matter (per cent):			
a. Calculated (Fleischman)	12.01	12.42	14.18
b. Weight analyses	11.86	12.18	13.96
In the ash:			
1 liter of milk contains (gram):			
CaO	1.582	1.579	1.911
MgO	0.035	0.121	0.173
K_2O	1.982	1.957	1.881
Na_2O	0.380	0.579	0.684
Cl	0.933	0.832	0.836
SO_3	0.192	0.235	0.303
P_2O_5	1.960	2.018	2.324
Total (ash without Fe_2O_3)	7.064	7.321	8.012
minus O corresponding to Cl	0.210	0.188	0.189
Net ash (without Fe_2O_3)	6.854	7.133	7.822

THE CHLORIDE-LACTOSE RELATION AS ONE OF THE MOST IMPORTANT BASES OF THE TASTE OF MILK

As has already been stated, those milk constituents which are naturally prominent in taste must be important in contributing to the primary taste of milk. The presence, as well as the quantity of these substances, is believed to be important.

It was expected that under otherwise similar conditions the highly dispersed substances, chlorides and lactose, with their blending properties would influence essentially the primary taste of the milk. Differentiation must be made between different conditions. A relatively high chloride-lactose number may be present in milk from normal cows advanced in lactation or it may result from a disturbance caused by udder bacteria.

In the first case one would have to consider the change in the milk as normal and the resulting change in milk taste would have to be considered as belonging to the primary taste. On the other hand, if the secretion has been disturbed by udder bacteria and the milk undergoes changes in its composition, then the chloride-lactose number may become still higher. Here one is dealing with a secondary influence on taste.

The milk from cows advanced in lactation which have a low bacterial content in their udders may show a pleasing primary taste in spite of the relatively high chloride-lactose number, since in some instances the lactose content is not essentially changed from normal. There is also no reason why an unpleasant secondary taste should be present in the milk which has undergone other slight changes in composition. Less objectionable are such cases then, since the increasing of the chloride-lactose ratio is to be considered as a result of a disturbance of secretion resulting from udder bacteria. As a rule the lactose content is materially lowered. It is to be expected that other substances, also affecting taste, will originate from the diseased udder tissues, as well as from udder bacteria present.

In this connection there is the further question as to the chemical combination of the chloride in milk. Soldner (4) and Porcher and Chevallier (5) have considered the chloride under

normal conditions as combined with sodium and potassium. Van Slyke and Bosworth (6) assume that the chloride of normally secreted cow's milk is united with the calcium. In this connection it would appear that a distinction must be made between the condition of the chloride which is present in the normally secreted milk and that which is secreted from abnormal udders. Whereas, the chloride does not need to be present with large amounts of sodium ions in all milks, it can probably be assumed with certainty that the chloride ion in salty milks, under abnormal conditions, is present with a relatively large amount of sodium ion. As far as the chloride of normally secreted milk is concerned, it has not been determined in which chemical combination it is present. Equal uncertainty exists as to the degree of ionization of the chlorides in the normally secreted milk. All of these questions are significant in attempting to determine the cause of certain tastes in milk.

In numerous publications (7), reference has been made to the salty and the salty-bitter tastes of milk, but the discussion of the chemical nature of this defect in taste has not been seriously considered. Gabathuler (8), in a study of the importance of lactose in nutrition, has pointed to the decrease of the lactose content in slight diseased conditions of the milk secreting tissues, while Hammer and Baily (9) in their studies on flavor, attached certain importance to the absence of alkali chlorides in milk. In the course of our own investigations the authors found that the chloride-lactose relation could regulate the foundation taste of milk to a certain degree. Milk samples with a high chloride-lactose ratio were judged less favorably than milk samples of like origin where the chlorine-lactose number was relatively low.

A possible explanation of the phenomenon is that for physiologically definite reasons the osmotic pressure of milk must be kept within certain narrow limits. Undoubtedly, the osmotic pressure of milk is largely dependent upon such substances as lactose in molecular dispersion, and the electrolytes, such as the alkali chlorides in ionic solution. The relation of salt to lactose may be due to the regulation of the osmotic pressure, in which case the chloride is taken from the blood or lymph, while

TABLE 3

The relation of chloride and lactose to taste of milk

NAME OF COW	FLAVOR SCORE	LACTOSE PERCENTAGE	CHLORINE AS CHLORIDE	CHLORIDE-LACTOSE NUMBER	JUDGES' COMMENTS
			grams per liter		
Felder	13.4	4.98	0.78	1.50	Slight sharp taste
Lisi	13.4	4.94	0.85	1.67	Pleasing taste
Müller	13.4	4.74	0.76	1.60	Very good taste
Müller	13.4	4.91	0.88	1.73	Even composition; very slightly sharp
Fuchs	13.4	4.90	1.10	2.18	Very good taste
Felder	13.3	5.23	0.76	1.39	Pleasant sweet taste; slightly off
Felder	13.3	4.86	1.02	2.02	Good milk
Müller	13.3	4.69	0.80	1.43	Even composition; good
Spiegel	13.2	5.06	1.01	1.93	Slight salty; taste not pleasing; even composition
Boni	13.2	4.70	1.24	2.59	Slight salty; composition off
Boni	13.2	4.54	1.24	2.60	Sharp aftertaste
Alice	13.2	5.08	0.77	1.45	Thin body
Lisi	13.1	4.91	0.88	1.67	Salty taste
Lisi	13.0	4.84	0.84	1.67	Aftertaste
Pfau	13.0	4.50	1.37	2.98	Slight aftertaste
Mei	13.0	4.64	1.08	2.26	Slight aftertaste; even composition
Miggi	13.0	4.24	1.42	3.27	Slight salty; aftertaste not pleasing
Gemse	12.9	4.19	1.31	3.10	Thin; watery; slight salty; slight aftertaste; sharp
Blösch	12.9	4.59	1.10	2.33	Composition off
Burgi	12.9	4.09	1.35	3.24	Bitter; salty
Schägg	12.8	4.74	0.94	1.81	Slight bitter aftertaste
Schägg	12.8	4.49	1.27	2.76	Astringent; aftertaste
Blösch	12.8	4.87	1.20	2.40	Sharp, sweet taste
Meisi	12.8	4.28	1.52	3.48	Slight salty
Gemse	12.7	4.19	1.28	2.98	Salty; off
Miggi	12.7	4.18	1.47	3.44	Slight salty
Rundi	12.6	3.74	1.73	4.54	Strong salty; aftertaste
Spiess	12.6	4.68	1.04	2.16	Disagreeable odor and taste; aftertaste
Schägg	12.5	4.77	1.13	2.30	Sharp; bitter
Spiri	12.3	4.82	0.95	1.90	Disagreeable taste
Gemse	12.3	4.22	1.35	3.12	Salty; aftertaste
Veiel	12.2	4.16	1.66	3.92	Salty; sharp (udder trouble)
Turk	11.5	4.47	1.44	3.15	Slight rancid odor and taste
Freude	11.0	4.48	1.24	2.48	Rancid odor and taste

the lactose regulation may probably result from a restraint in the percentage of lactose elaborated. The relation between chloride and lactose and the taste scores of the different milk samples may not correspond in all cases. As previously emphasized, there are instances where a defect in taste of a secondary nature may be present in addition to a high chloride-lactose ratio. The chloride-lactose relation is only one of the factors of importance in the analytical determinations of taste in cow's milk.

The value of the chloride-lactose number is identical with the chlorine-lactose number, as described by Koestler (3) and is found by multiplying by 100 the ratio of percentage chloride to percentage lactose. The chlorides reported in grams per liter in column 4 of table 3 have been changed to percentage in making the calculation to determine the chloride-lactose number.

It is apparent that the samples of milk with the higher scores on flavor had a relatively low chloride-lactose number. In all but one instance, the salty taste was accompanied by a chloride content above 1 gram per liter.

THE TASTE OF SOLUTIONS CONTAINING LACTOSE AND SODIUM CHLORIDE

In further study of the sources of the taste in milk and the influence of lactose and sodium chloride upon the taste, solutions containing lactose and sodium chloride were prepared with distilled water. The solutions contained the maximum and minimum lactose content likely to be found in milk and the corresponding chloride content. The concentration of the solutions was made to correspond with the osmotic pressure of normal milk, considering for this study that the osmotic pressure would be caused only by these two substances.

The results indicated that in warm solutions (35°C.) the taste of the salt and sugar are blended and a more pleasing taste is given than in the cold solutions (15°C.). The sugar taste seemed more apparent in the warm solutions, while the salty taste seemed more prominent in cold solutions. In the cold solutions the sugar and salt tastes seemed to be separate and were less pleasing.

The influence of the temperature upon the taste of these solutions was found to vary according to the relative concentrations of sugar and salt in the solutions.

THE INFLUENCE OF SODIUM CHLORIDE AND CALCIUM CHLORIDE ON THE TASTE AND ODOR OF MILK

Although the question as to the combination of chloride in normal milk must remain open, it seemed to be of interest to compare the influence on the taste of milk of the addition of equivalent amounts of sodium chloride and calcium chloride. For this purpose increasing amounts of sodium chloride and calcium chloride were added to different samples and they were subjected to examination for taste and odor. The results are tabulated in table 4.

When a sufficient quantity of sodium chloride was added to the milk, a salty taste resulted. The samples to which calcium chloride had been added did not give a salty taste, but the milk produced an astringent effect in the mouth. An addition of calcium chloride equal to 0.355 gram of calcium oxide per liter of milk produced a pronounced sharp, astringent taste.

When adding sodium chloride to Felder's milk to bring the content of sodium chloride to 0.1539 per cent, a weak, salty taste was present in the milk. The salty taste produced by adding a given quantity of sodium chloride was less prominent, however, than the salty taste of secreted milk with the same sodium chloride content.

It is interesting to note that the added sodium chloride and calcium chloride in certain amounts produced an odor in the milk. These salts probably change the physico-chemical properties of the milk in such a way that the milk is not able to retain certain gases which gives the impression of odor.

RELATION OF FATS AND PROTEIN TO THE TASTE OF MILK

When beginning the study of the cause of variations in the taste of normal milk, attention was naturally given to the influence of butterfat on the taste of normal milk. Several lots of mixed milk were passed through a cream separator and the taste of the

skim milk compared with that of the original whole milk. Different samples gave slightly different impressions, but in general

TABLE 4

The relation of sodium chloride and calcium chloride to milk flavor

COW	SCORE OF NORMAL MILK	MIXTURES USED	NaCl ADDED*	FLAVOR SCORE	REMARKS	$CaCl_2$ ADDED*	FLAVOR SCORE	REMARKS
			grams			grams		
Lisi	13.2	I	0.0349	13.3	Good taste	0.03516	13.0	More flavor but aftertaste
		II	0.0698	12.9	Salty, sharp, slightly astringent	0.07032	12.9	Not pleasing taste, astringent
		III	0.1047	12.6	Distinctly salty	0.10548	12.8	Aftertaste, off, astringent
		IV	0.1396		Objectionable odor, distinctly salty			Stable-like odor, distinctly off, astringent
Müller	13.5	I	0.0349	13.0	Slightly astringent, very slight salty	0.03516	13.2	Slight odor, slightly astringent
		II	0.0698	13.0	Slight salty	0.07032	13.1	Slightly astringent
		III	0.1047	12.7	Salty, sharp	0.10548	12.8	Distinctly astringent
		IV	0.1396	12.2	Odor, distinctly salty	0.14064	12.2	Odor, off, astringent
Felder	13.1	I	0.0349	13.2	Better than original	0.03516	13.2	Astringent
		II	0.0698	13.0	Not astringent, slight salty	0.07032	12.8	Distinctly astringent, sharper than normal
		III	0.1047	12.7	Odor, salty	1.10548	12.5	Odor, astringent, strong, sharp

* Added to 100 cc. of milk.

when the whole milk had a pleasing taste, smooth body, and was even in composition, the skim milk was equally pleasing in primary taste, was not so full but was nearly as palatable as the

whole milk. This supports the previous statement in this paper that the fat is not an important constituent in milk influencing the primary taste.

THE DEGREE OF DIALYSABILITY OF THE MILK INGREDIENTS AND ITS INFLUENCE UPON THE PRIMARY TASTE OF THE MILK

It was assumed from the beginning that the different milk ingredients participate in a different degree in bringing about the

TABLE 5

The influence of dialysis on the taste of milk

COW	ORIGINAL MILK	TASTE OF MILK IN COLLODION SACK	MILK USED	WATER USED
			cc.	cc.
Lisi	Good taste, sweet, slightly thin body, score—13.4	Almost tasteless, flat, watery	120	200
Felder	Pleasing taste, very slightly watery	Almost tasteless, flat, lacked flavor, watery taste, and body almost like water	120	120

TABLE 6

The chemical analysis of one of the milk samples subjected to dialysis

ANALYSES MADE	ORIGINAL MILK	DIALYZED RESIDUE IN COLLODION SACKS	DIALYZATE IN WATER SOLUTION
Specific weight	1.0306	1.0168	1.009018
Fat percentage	3.69	3.30	
Dry matter percentage	12.32	8.60	2.10
Milk sugar percentage	4.76	2.31	1.73
Chloride—grams per liter		0.412	0.195
Ash percentage		0.0433	0.0372
Phosphoric acid		0.110	0.022
Calcium oxide percentage		0.112	0.024

primary taste of the milk. It is furthermore reasonable to assume, for this purpose, that the degree of dispersity of the milk ingredients is an important item.

In an effort to determine the correctness of these assumptions,

the milk of different cows was subjected to dialysis by using the collodion filter with a fifteen hour dialysis against distilled water held at a temperature below 15°C. Table 5 gives the results of the examination of two samples which were typical of several milks similarly treated, and table 6 includes the chemical analyses of the original milk, the dialyzed residue and the dialyzate.

In order to have an exact comparison between the taste of the original milk and that of the dialyzed residue, the sample of Lisi's milk referred to in table 5 was brought to the same water content as the dialyzed residue which had been diluted during dialysis, with water drawn through the membrane.

It was manifest that the milk which was partially freed from the easily dialyzed materials was almost tasteless. At the time of tasting, the impression given was that of tasting viscous water, whereas the original milk which had been diluted to the same water content still gave essentially the taste of the original milk.

With these simple dialyzing experiments, proof is given that along with the partial removal of the dialysable substances of the milk by far the greatest part of the primary taste is also eliminated from the milk; or, in other words, the primary taste of the milk is to the greatest extent associated with the finely dispersed, easily dialysable ingredients; whereas, fat and milk protein, both of which remain almost entirely within the dialyzed residue, are concerned, if at all, to only a very slight degree with the primary taste of the milk.

It can furthermore be determined by dialysis that certain elements of the secondary taste remain in the dialyzed residue. This is evident for the reason that these elements of taste are combined in some manner with the substances (fat and protein) which can be dialyzed only with difficulty.

DISCUSSION

Certain constituents are capable of contributing to the taste of milk. Others do not produce definite taste reactions, but they do influence the taste impression by their influence upon the physical properties of the milk. Acids, because of the large quantity of hydrogen ions present, give a more prominent taste

impression than do more weakly ionized materials, such as albuminoids.

Not all of the taste producing ingredients of milk give the impression of taste to the same extent nor at the same time. The water or saliva solubility of the separate ingredients is undoubtedly concerned, since the more soluble substances can be expected to give rise to the impression of taste under similar conditions sooner than do the incompletely dispersing ones.

The degree of dispersion of the milk components influences the total taste impression. Since the fat has a relatively low degree of dispersity and shows marked absorptive qualities, it may become a carrier of those flavor producing materials which are absorbed into the milk.

The degree to which the different taste impressions can be identified during the act of tasting is not an indefinite matter in the judging of milk taste. As soon as the milk has been taken into the mouth, generally those taste sensations predominate which originate from the substances in true solution, such as lactose and salts. Lactose and certain salt ingredients take an important part in the fundamental taste of the milk.

The foundation taste of milk resulting from its natural composition, is supplemented by certain secondary tastes. These give definite impressions such as acid and bitter tastes, or they represent a mixture of a non-typical sort, such as feed or stable flavor. Such secondary flavors are probably to be considered generally as "foreign" to normal milk.

The secondary flavor may very often be of a volatile, aromatic nature, and hence show itself more or less plainly in the odor of the milk. There are also secondary flavor sensations which can be classified as "aftertaste," and which evidently become concentrated upon the mucous membranes. They contribute to the sense of taste, but do not leave a distinct impression until the greater part of the milk has been removed from the mouth.

With the uncertainty which still exists concerning the identification of the different degrees of taste, it is possible to properly designate the finer sensations of taste to only a limited degree. However, one is able to think of the taste of milk as it is influenced

by its constituents. As methods for the more complete analyses of milk are perfected, it should become possible to determine, to a still greater extent, the relation of the constituents of milk to its taste.

It is recognized that many phases of this subject remain to be further investigated. It is expected that others may be stimulated to contribute to this important, but hitherto neglected, field of study. The senior author is continuing research in this subject.

SUMMARY

1. The taste of milk, as this word is commonly used, is the sensation perceived when milk is taken into the mouth. The term "flavor," as used in this paper, is a combination of the sensations of taste, perceived in the mouth, with those of smell, produced through the medium of the inner nasal passages. It has aided in precision to confine the term "taste" to those sensations which are perceived only in the mouth.

2. The primary taste of milk has been designated as the sum of all of the taste impressions coming from normal milk, and not influenced by feed or the secretion of abnormal milk.

3. The secondary taste includes that which is added to the primary taste from different sources, such as feed and products of disease. It is necessary to eliminate these secondary influences in order that primary tastes can be properly determined.

4. The chloride-lactose relation is one of the most important bases of milk taste. Milk samples with a high chloride-lactose ratio were judged less favorably than those of like origin where the chloride-lactose ratio was relatively low.

5. The primary taste of skim milk is practically equal to that of the whole milk from which it is separated.

6. By the application of dialysis, it was possible to separate the milk into two parts (dialyzate and residue) with extreme differences in taste. It was found that nearly all of the milk components producing the primary taste were present in the dialyzate, while the components remaining in the residue could be designated as free from taste.

7. By dialysis it has been further demonstrated that fat and protein substances, as well as certain difficultly dialyzable salt components, which go to make up a large percentage of the milk content, take only a subordinate part in the primary taste of the milk.

8. The dialysis of milk containing a pronounced feed taste has shown that the feed taste was found very largely in the residue, and appeared to be either not dialysable or in some way combined with milk fat or with other non-dialysable materials.

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STUDIES ON THE NUTRITIVE VALUE OF MILK

III. THE SUPPLEMENTARY VALUE OF VARIOUS CONSTITUENTS OF SYNTHETIC BASAL RATIONS*

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It has previously been reported that rats, started as weanlings, could not be raised to maturity on an exclusive whole milk diet (1) (2). This failure was attributed to the development of nutritional anemia, due to an insufficient intake of copper and iron. Inasmuch as rats had been successfully raised on synthetic rations, the problem of discovering the component or components of such a ration that would adequately supplement milk presented itself. With this object in mind, the following experiment was undertaken.

EXPERIMENTAL PROCEDURE

Albino and piebald rats were fed a basal ration of liquid whole milk to which was added one of the constituents of ordinary synthetic basal rations, such as casein, agar, starch, yeast, salts, cod liver oil, wheat germ oil, gelatin, and rice polishings. This procedure resembles somewhat that followed by Mattill and coworkers (3), but these investigators were concerned primarily with reproduction. Since vitamin D may be considered a constituent of a basal ration, one group of rats was fed milk irradiated in a thin layer under a Cooper-Hewitt lamp for ten minutes at a distance of 14 inches from the burner. Two male and two female rats were placed together in a cage in order to allow every opportunity for reproduction to occur. The amount of each supplement fed, arbitrarily chosen, was weighed or measured each day and added to the milk. This was thoroughly stirred in glass beakers and placed in the cages in the afternoon. The amount of milk fed at this time was small enough to insure complete consumption overnight. In the morning more milk was added to

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TABLE 1

Gain in weight and hemoglobin content of the blood of rats fed milk only, and milk plus various supplements

RAT NUMBER	INITIAL WEIGHT	AFTER 2 MONTHS		AFTER 3 MONTHS	
		Weight	Hemoglobin	Weight	Hemoglobin
Milk exclusively (ad lib.)					
	grams	*grams*	*grams per 100 cc.*	*grams*	*grams per 100 cc.*
E135 ♂	59	90	1.38–	*	*
E136 ♂	60	95	1.38–	Dead	
E137 ♀	64	95	1.38–	*	*
E138 ♀	58	96	2.34	Dead	
Milk plus dried yeast (0.4 gram per rat)					
E139 ♂	61	155	16.52	211	16.52
E140 ♂	62	183	15.84	233	15.84
E141 ♀	62	123	16.52	152	16.52
E142 ♀	60	148	13.77	172	13.77
Milk plus cod liver oil (3 drops per rat)					
E143 ♂	63	137	2.07	Dead	
E144 ♂	61	Dead			
E145 ♀	61	85	2.75	Dead	
E146 ♀	56	86	2.62	Dead	
Milk plus McCollum's salt mixture 185 (0.25 gram per rat)					
E155 ♂	60	93	8.68	134	13.08
E156 ♂	61	152	12.39	177	11.70
E157 ♀	56	117	16.52	126	16.52
E158 ♀	62	117	12.39	137	12.67
Milk plus gelatin (0.25 gram per rat)					
E167 ♂	52	150	2.75	169	2.34
E170 ♂	54	130	2.34	Dead	
E168 ♀	52	138	3.86	150	3.86
E169 ♀	49	Dead			
Milk plus agar (0.50 gram per rat)					
E175 ♂	52	180	9.36	214	7.57
E178 ♂	52	160	6.75	170	9.36
E176 ♀	50	133	8.12	135	4.96
E177 ♀	49	128	7.16	135	10.33

TABLE 1—*Concluded*

RAT NUMBER	INITIAL WEIGHT	AFTER 2 MONTHS		AFTER 3 MONTHS	
		Weight	Hemoglobin	Weight	Hemoglobin
Milk plus casein (2 grams per rat)					
	grams	*grams*	*grams per 100 cc.*	*grams*	*grams per 100 cc.*
E180 ♂	64	174	13.08	190	15.15
E182 ♂	52	195	13.77	205	11.15
E179 ♀	59	134	13.77	145	16.25
E181 ♀	49	138	15.15	Died from loss of blood	
Milk plus starch (2 grams per rat)					
E185 ♂	51	175	8.26	218	10.60
E186 ♂	49	157	7.30	.90	11.02
E183 ♀	50	130	8.40	158	10.88
E184 ♀	49	135	7.57	158	13.77
Milk plus rice polishings (0.4 gram per rat)					
E247 ♂	51	144	3.17	168	2.62
E249 ♂	54	146	2.34	150	1.79
E246 ♀	51	117	5.23	129	5.92
E248 ♀	53	Dead			
Milk plus wheat germ oil (3 drops per rat)					
E251 ♂	62	Dead			
E253 ♂	58	88	1.38	Dead	
E250 ♀	55	65	3.03	Dead	
E252 ♀	48	Dead			
Milk, irradiated (ad lib.)					
E238 ♂	52	72	1.38	Dead	
E240 ♂	50	71*	1.38*	*	*
E239 ♀	52	89	2.48	Dead	
E241 ♀	47	106*	1.38*	*	*

* Yeast added.

the beakers, so that the consumption of milk was ad libitum. Weights were recorded weekly and hemoglobin determinations were made on one rat of each group each week. The technique of obtaining blood samples and of determining hemoglobin has been previously described (2).

RESULTS

The results are presented in table 1.

It is very evident that yeast, McCollum's salt mixture 185, agar, casein and starch were helpful in preventing anemia. Of these substances, yeast was by far the most effective, the hemoglobin of the rats receiving it remaining at a high level throughout. In the case of the other supplements there was a drop in hemoglobin during the critical period, but as the rats matured there was gradual regeneration.

A special word of explanation concerning the yeast used needs to be made at this point. The sample was a dry, starch-free powder, originally purchased for use as a source of vitamin B. The amount fed, 0.4 gram, had been found to be adequate to

TABLE 2

Iron and copper content of supplements, and daily intake

MATERIAL	IRON	COPPER	DAILY INTAKE	
			Fe	Cu
	per cent	*per cent*	*mgm.*	*mgm.*
Yeast	0.0918	0.0032	0.49	0.04
Salts 185	0.2561		0.76	0.03
Agar	0.0175		0.21	0.03
Casein	0.0162	0.0024	0.44	0.07
Starch	0.0135	0.00073	0.39	0.04

allow normal growth on an otherwise vitamin-B-free diet. The results obtained with this yeast, both as a curative and as a preventive of nutritional anemia, were so successful that two other samples from the same company, and seven samples from different companies, were assayed, for their anti-anemic potency. Each sample was found to be decidedly inferior to the first one tried. This is emphasized here because it points out the lack of uniformity that may exist in even a high-grade product, and also it suggests a precaution to be taken when formulating a basal ration to be used in connection with iron metabolism studies.

In order to find a possible explanation of the behavior of these substances in preventing anemia, determinations were made of

their copper and iron content. Copper was determined by the Biazzo method (4) and iron by the Fowweather (5) method, except in the case of the salt mixture where the Elvehjem and Lindow method for copper (6), and the Elvehjem and Hart method for iron (7) were used. These figures are presented in table 2.

This indicates a rather definite relationship between the amount of copper and iron required by the rat for normal hemoglobin formation. Wherever at least 0.45 mgm. of iron, and 0.04 mgm. of copper were furnished, the hemoglobin level was highest, as in the case of yeast and casein. Hart, Steenbock, Waddell and Elvehjem (8) secured excellent results through the administration six times per week of 0.05 mgm. of copper and 0.5 mgm. of iron.

DISCUSSION

The exceptional response obtained through supplementing milk with 0.4 gram of yeast daily may be attributed to an adequate intake of copper and iron. The amounts of copper and iron supplied were probably optimum, the ratio between these two elements being 1:12.25. That no organic factor was involved was shown when the ash of 0.4 gram of yeast was used with equally good results. Evidently vitamin B was no factor as negative results were obtained with rice polishings. The same applies to vitamins A, D, and E, as cod liver oil, irradiated milk and wheat-germ oil likewise exerted no influence. Cartland and Koch (9) concluded that vitamins A, B, and E probably were not essential for hemoglobin formation in the rat. These same investigators found that casein promoted hemoglobin synthesis, as did McCay (10).

The weights at two and three months (table 1) indicate that when rats are given whole milk ad libitum, the only deficiency exists in the mineral portion of the diet. When additional protein and energy in the form of casein and starch, respectively, were added, no increase in the rate of growth occurred. Of the vitamins, B and E might have been slightly deficient. The inferior growth of rats receiving the salt mixture, however, points

to a mineral deficiency. The rats in this group received an adequate supply of iron as iron citrate, but their intake of copper was inadequate. The addition of bulk in the form of agar had no effect on the rate of growth and was not very effective in preventing anemia because at the level fed the total intake of copper and iron was insufficient.

Yeast, casein, agar, starch and McCollum's salt mixture 185 are often used in synthetic basal rations. When iron metabolism, or hemoglobin regeneration studies are conducted, and a basal ration containing any of the above ingredients is contemplated, a careful evaluation of both the copper and iron content of the diet should be made.

SUMMARY

Yeast, casein, starch, agar, and McCollum's salt mixture 185 were found effective in preventing nutritional anemia in rats fed an exclusive whole milk diet. The degree of effectiveness was found to depend in general upon the amount of copper and iron furnished by each substance.

Cod liver oil, gelatin, rice polishings, wheat germ oil, and irradiated milk were found to be ineffective in preventing nutritional anemia in rats.

An exclusive whole milk diet fed ad libitum to rats furnishes adequate protein, energy, and vitamins for growth, but is deficient in copper and iron.

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THE PHYSIOLOGICAL EFFECT OF RATIONS RESTRICTED PRINCIPALLY OR SOLELY TO THE ALFALFA PLANT

I. THE CALCIUM, PHOSPHORUS AND NITROGEN METABOLISM OF DAIRY CATTLE*

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Due to the rather common practice in many of our western alfalfa producing sections of feeding dairy cattle exclusively or almost so on alfalfa, the Department of Dairy Husbandry of the Oregon Experiment Station (1) has been conducting practical alfalfa feeding trials in an attempt to determine the economy of this practice and the extent to which alleged regional differences exist in the quality of alfalfa hay. During the summers of 1926[1] and 1927 animals from this group were placed in metabolism stalls and their calcium, phosphorus and nitrogen balances were determined for periods during which the daily milk yields varied from approximately 9 to 18 kgm. In these metabolism studies, we fed alfalfa hay with and without supplements of starch, linseed oil meal, or di-sodium phosphate.

The study of rations restricted to alfalfa is of peculiar interest in extensive regions of the West where alfalfa is grown so abundantly and cheaply that the relatively high price of concentrates has led to the frequent practice of restricting dairy cattle very

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[1] The 1926 trials were started and partially completed while the nutrition work in the Department of Agricultural Chemistry was in charge of Dr. H. G. Miller. A large share of the analytical work reported in this paper was done with the assistance of Mr. H. M. Shepardson.

largely to alfalfa. Furthermore, this problem is of interest to the nutrition worker because it deals with an attempt to determine what substances, in relatively small amounts, are needed to make a fairly well balanced ration from a common leafy leguminous plant. Among the factors to be considered in rations restricted largely to alfalfa hay are: their net energy value, the biological value of alfalfa proteins, the phosphorus content of alfalfa, and the fact that alfalfa may contain anywhere from about 3 to 25 times as much calcium as phosphorus. The last mentioned fact appears to have been almost entirely overlooked by nutrition workers, as has also the fact that alfalfa hay frequently contains less than 0.20 per cent phosphorus.

It is not our purpose here to review the extensive literature dealing with experiments in which alfalfa has been fed as a constituent of more or less balanced rations. Experimental data dealing with alfalfa hay as the sole or principal constituent of rations for dairy cattle are limited, presumably because the practical aspects of this problem have not impressed most investigators. Attention, however, is called to the work of Woll (2) and to the discussions of Eckles (3) and of Henry and Morrison (4), dealing with the economy of alfalfa hay feeding. It should be kept in mind that the problem varies with the quality of hay and with the amount of alfalfa which is fed in the green state.

THE 1926 METABOLISM TRIALS

In these trials we fed second cutting alfalfa hay with and without supplements of starch or linseed oil meal. The hay was taken from the same lots as that used in the practical feeding trials and had been selected as being representative of the regions in which it was grown. This hay (except that fed to cow 629) was grown under irrigation in eastern Oregon and was cut when in about one-half bloom. It was clean and well cured but was slightly coarse. Cow 629 was fed quite a coarse grade of hay grown in the Willamette Valley. The nitrogen content of the eastern Oregon hay varied from 1.58 to 1.71 per cent; the calcium content from 0.92 to 1.03 per cent; and the phosphorus content

TABLE 1

Description and history of the cows used in the 1926 metabolism trials

COW NUMBER	BREED	APPROXIMATE AGE	LACTATION PERIOD	PREVIOUS FEEDING HISTORY
222	Holstein	8	Fifth period began May 13, 1926	On alfalfa hay from February 1, 1926
468	Ayrshire	3½	Second period began March 22, 1926	On alfalfa hay from February 1, 1926
623	Guernsey	6½	Fifth period began March 10, 1926	On alfalfa hay from April 7 to July 8, 1924. On alfalfa hay from January 24, 1925
629	Guernsey	5	Third period began March 10, 1926	On alfalfa hay from February 1, 1926

TABLE 2

Average daily metabolism data secured from 1926 trials

COW NUMBER	DATE	DISTINGUISHING FEATURES OF RATIONS USED	MILK YIELD	NITROGEN Feed Milk Excreta Balance	CALCIUM Feed Milk Excreta Balance	PHOSPHORUS Feed Milk Excreta Balance
			kgm.	*grams*	*grams*	*grams*
623	April 7–17	13.40 kgm. alfalfa hay	12.57	226.6	132.9	23.5
				65.5	17.1	11.1
				166.8	88.6	14.0
				−5.7	+27.2	−1.6
468	April 7–17	14.26 kgm. alfalfa hay	16.58	240.8	142.7	25.0
				88.0	16.5	11.6
				178.2	109.9	14.4
				−25.4	+16.3	−1.0
623	May 14–24	9.40 kgm. alfalfa hay; 2.72 kgm. starch	9.29	161.0	97.0	16.5
				41.8	11.8	10.4
				125.6	98.8	16.2
				−6.4	−13.6	−10.1
468	May 14–24	11.75 kgm. alfalfa hay; 2.72 kgm. starch	14.52	201.2	121.0	20.6
				69.2	16.0	13.8
				145.7	76.9	19.6
				−13.7	+28.1	−12.8

from 0.162 to 0.175 per cent. The nitrogen content of the Willamette Valley hay varied from 1.26 to 1.54 per cent; the calcium content from 0.81 to 1.14 per cent; and the phosphorus content from 0.185 to 0.212 per cent. The nitrogen content of the hay fed to cow 629 during the metabolism trials proved to be unexpectedly low. Previous analysis of samples taken from the

TABLE 3

Average daily metabolism data secured from 1926 trials (Continued)

COW NUMBER	DATE	DISTINGUISHING FEATURES OF RATIONS USED	MILK YIELD	NITROGEN Feed Milk Excreta Balance	CALCIUM Feed Milk Excreta Balance	PHOSPHORUS Feed Milk Excreta Balance
			kgm.	*grams*	*grams*	*grams*
222	June 21–July 1	16.66 kgm. alfalfa hay	18.80	253.9 77.5 193.2 −16.8	147.0 13.4 111.1 +22.5	26.6 14.7 22.0 −10.1
629	June 21–July 1	11.28 kgm. alfalfa hay	10.07	139.5 52.6 119.0 −32.1	85.3 8.0 97.7 −20.4	26.7 11.9 16.2 −1.4
222	August 25–September 4	17.90 kgm. alfalfa hay; 1.36 kgm. linseed oil meal	14.82	347.7 68.3 293.0 −13.6	163.1 14.3 145.4 +3.4	41.1 11.7 35.3 −5.9
629	August 25–September 4	12.84 kgm. alfalfa hay; 1.36 kgm. linseed oil meal	8.12	265.4 43.8 213.0 +8.6	146.7 8.4 109.8 +28.5	35.8 9.0 32.4 −5.6

same lot from which this hay was obtained had shown a nitrogen content similar to that of the eastern Oregon hay. The crude fiber content of the above hays varied from 30.0 to 33.0 per cent.

The cows were placed in metabolism stalls and subjected to a preliminary feeding period while becoming accustomed to the daily routine involved in metabolism trials. The mixed excreta were collected, weighed and sampled daily. As a general rule, we conducted our work in units of five days each in order to avoid

the chance of losing the results of an entire period. While we report our results for the entire period, it may be remarked that we occasionally found the results of a five-day period out of line, indicating that even for such a uniform ration as chopped alfalfa hay, metabolism periods should be of at least ten days duration.

The cows used in the 1926 trials are briefly described in table 1. The results obtained with two cows fed alfalfa hay with and without the addition of starch are reported in table 2; those obtained with two cows fed alfalfa hay with and without the addition of linseed oil meal, in table 3.

Upon examining the balance data in tables 2 and 3, we were at once impressed with the fact that the phosphorus balances were always negative and the nitrogen balances usually so. In view of the high calcium intake one would expect to find the calcium balances positive, although it is not clear why they should bear so little apparent relation to the losses of phosphorus from the body.

The supplements of starch and linseed oil meal did not lead to results from which definite conclusions can be drawn. The feeding of starch resulted in a decreased consumption of hay, while the feeding of linseed oil meal was accompanied by a slight increase in hay consumption. We are therefore unable, from these experiments, to draw any definite conclusions concerning the effect of an increase in total digestible nutrients over those contained in rations restricted to alfalfa hay, nor are we able to make any definite statements concerning the value of supplementing alfalfa hay with linseed oil meal proteins, since the latter were added to an already high level of protein intake and could not therefore be expected to show marked results unless the supplementary effect was very pronounced.

A comparison of the actual hay consumption and milk yields with the commonly accepted feeding standards, compels us to come to the conclusion that the intake of total digestible nutrients and of phosphorus can not be considered adequate for more than very moderate milk production. The faulty nitrogen assimilation may be related to one or more of a number of factors, among

which are the energy and protein intakes, the biological value of alfalfa protein, a possible relationship between phosphorus and protein metabolism and the general suitability of rations restricted to alfalfa hay. In the case of the 1926 trials the low protein content of the hay undoubtedly was an important factor contributing to the negative nitrogen balance.

During the course of the above experiments, cows 468 and 623 lost approximately 25 to 30 pounds in live weight. Cows 222 and 629 suffered somewhat greater losses in weight during the periods on alfalfa hay only, but remained practically constant in weight during the periods when alfalfa hay was supplemented with linseed oil meal. The losses in weight referred to are considered as being incidental to the stage of lactation and quite independent of the various supplements used in our balance trials. It has been our experience that cows fed exclusively on alfalfa hay begin their lactation period in good condition with a fairly liberal milk flow, which is followed by a sharp decline in milk flow and a moderate loss in body weight.

THE 1927 METABOLISM TRIALS

On the basis of the preliminary results secured from the 1926 metabolism trials and certain theoretical considerations, such as the significance of the Ca/P intake ratio, we outlined in 1927 a more extensive program for the study of rations restricted principally or solely to alfalfa. The 1927 trials were conducted as part of a series which we hope will throw some light on the question of the significance of the Ca/P intake ratio in the nutrition of dairy cattle. No extensive search of the literature is necessary to show that alfalfa hay may contain anywhere from about 3 to 25 times as much calcium as phosphorus, although under ordinary conditions this ratio is more likely to be around 5 to 8. For example, Scott (5) has recently called attention to a sample of alfalfa hay which contained 28 times as much calcium as phosphorus.

The 1927 trials were conducted in much the same manner as those in 1926. Two cows were fed alfalfa with and without the

addition of di-sodium phosphate ($Na_2HPO_4 \cdot 12H_2O$). After a preliminary feeding period in the metabolism stalls, a ten-day balance was run with alfalfa only, followed by an intermediate period of four days, during which the sodium phosphate was fed in amounts increasing from 200 to 400 grams daily. During the next ten days 400 grams were fed daily. The behavior of the cows in the stalls and the collection of excreta were entirely satisfactory. We made a practice of exercising the cows about 15 minutes each day while the excreta were being weighed, mixed and sampled.

The hay used in these trials was second cutting grown on non-irrigated land. It was cut at about one-fourth bloom, left in the

TABLE 4

Description and history of cows used in the 1927 metabolism trials

COW NUMBER	BREED	APPROXIMATE AGE	LACTATION PERIOD	PREVIOUS FEEDING HISTORY
		years		
456	Ayrshire	5½	Fourth period began May 5, 1927	On alfalfa hay from January 24, 1925
462	Ayrshire	5½	Fourth period began April 28, 1927	On alfalfa hay from January 24, 1925

swath for one-half day, in the windrow for one-half day, shocked for 7 days and stacked. The hay was graded as U. S. No. 1, and was finer in texture and more leafy than that used in the 1926 trials. This hay contained 2.01 per cent nitrogen, 1.37 per cent calcium, 0.174 per cent phosphorus, and 27.6 per cent crude fiber. The cows used in these trials are briefly described in table 4.

In table 5 are given the data secured in the 1927 trials. We have included the four-day intermediate period because it forms a part of the picture of what took place during this period. In contrast with the 1926 trials, the nitrogen balances were positive. This appears to have been due to the difference in nitrogen content of the hays used. The 1926 hay analyzed from approximately 1.3 to 1.7 per cent nitrogen, while that of 1927 analyzed

2.0 per cent nitrogen. The calcium balances were again positive. As before, when alfalfa alone was fed, the phosphorus balances

TABLE 5

Average daily metabolism data secured from 1927 trials

COW NUMBER	DATE	DISTINGUISHING FEATURES OF RATIONS USED	MILK YIELD	NITROGEN Feed Milk Excreta Balance	CALCIUM Feed Milk Excreta Balance	PHOSPHORUS Feed Milk Excreta Balance
			kgm.	*grams*	*grams*	*grams*
456	July 7–17	14.18 kgm. alfalfa hay	15.61	284.7 66.6 214.3 +3.8	194.3 15.3 172.0 +7.0	24.6 12.8 15.9 −4.1
462	July 7–17	14.23 kgm. alfalfa hay	12.59	286.5 56.6 209.7 +20.2	195.5 14.2 173.3 +8.0	24.8 10.4 16.2 −1.8
456	July 17–21	13.57 kgm. alfalfa hay; 200, 300, 390 and 400 grams, respectively of di-sodium phosphate	14.64	272.5 62.5 193.3 +16.7	186.0 15.0 157.0 +14.0	52.3 11.5 24.0 +16.8
462	July 17–21	13.42 kgm. alfalfa hay; 200, 300, 395 and 400 grams, respectively of di-sodium phosphate	12.24	271.3 52.0 203.8 +15.5	184.8 13.8 151.3 +19.7	52.0 9.8 23.8 +18.4
456	July 21–31	13.43 kgm. alfalfa hay; 400 grams daily of di-sodium phosphate	13.94	269.9 56.3 202.4 +11.2	183.9 15.3 164.5 +4.1	58.4 11.1 46.0 +1.3
462	July 21–31	13.57 kgm. alfalfa hay; 400 grams daily of di-sodium phosphate	10.81	273.3 46.1 204.1 +23.1	186.6 11.8 167.4 +7.4	58.8 8.5 48.4 +1.9

were negative. When, however, di-sodium phosphate was fed, slightly positive balances were obtained. We realize that the

differences between the negative balances on hay alone and the positive balances when sodium phosphate was fed, are not large, but they occurred so consistently that they appear to be significant.

Attention is called to the data during the four-day intermediate period. It will be noted that the phosphorus lag in the digestive tract shows up very nicely in an apparently high positive balance, and that along with it there is evidence of an associated lag in the excretion of calcium. As would be expected, the nitrogen balance data are not affected in this manner. Our results are in agreement with those of Meigs et al. (6) in that they indicate about a two-day lag in the excretion of phosphorus.

A DISCUSSION OF THE SIGNIFICANCE OF OUR RESULTS

It is difficult to determine to what extent dairy cattle restricted largely to alfalfa hay are merely suffering from under-feeding due to the bulky nature of the ration and to what extent specific nutritive factors are involved. We are reasonably certain that production must often be limited by the intake of total digestible nutrients, and in the case of low protein hay, by the protein intake. The milk production of the animals restricted to alfalfa hay was approximately one-half that to be expected on the regular herd ration. When hay of unusual quality and green alfalfa are fed, it is reasonable to expect somewhat higher production. It is our feeling that there is still a great deal to be learned concerning the factors which determine "quality" of hay.

Our data on nitrogen assimilation do not warrant any definite conclusions concerning the biological value of alfalfa protein. We were at first inclined to interpret the negative nitrogen balances obtained during 1926 as evidence of a lack of protein quality, but our 1927 data do not necessarily support such an interpretation. We (7) are, however, accumulating evidence with rats which shows that alfalfa protein (as supplied by finely ground leaves at a level of 12 to 15 per cent crude protein) does not constitute an adequate source of cystine.

Standards for the phosphorus requirements of dairy cattle are so limited that it is difficult to definitely evaluate rations restricted

largely to alfalfa from the point of view of their phosphorus content. The regularity with which we obtained negative phosphorus balances on alfalfa hay, however, indicates that the phosphorus content of alfalfa hay can not be considered adequate for more than moderate milk production. We consider it worthwhile to emphasize the fact that alfalfa hay may often contain less than 0.20 per cent phosphorus, and therefore should not be regarded as a phosphorus-rich feed. Although we have some evidence originating with practical dairymen, indicating a phosphorus deficiency among animals restricted largely to alfalfa, the situation in Oregon does not appear to have reached an acute stage. It is our opinion that this is true largely because even most well-bred animals lose their impulse to secrete milk to a sufficient extent to protect their body stores of phosphorus against loss during most of their lactation cycle.

The data presented in this paper do not indicate to what extent phosphorus retention is conditioned by the high Ca/P intake ratios incident to rations restricted largely to alfalfa. Meigs and coworkers (6) have expressed the opinion that there is some evidence which indicates that rations for dairy cattle should preferably contain not more than two times as much calcium as phosphorus. In a later paper (8) it is suggested that "the amount of calcium probably should not exceed the amount of phosphorus by more than 50 per cent." While general considerations compel us to regard extremely high Ca/P intake ratios with suspicion, we must admit that our present data may also be interpreted as indicating that such ratios may be quite high without producing nutritional disaster.

In addition to the metabolism trials reported in this paper, we conducted another series during the summer of 1928, again using cows 456 and 462. We repeated the feeding of alfalfa hay with and without di-sodium phosphate, and, in addition, we fed alfalfa hay with supplements of calcium carbonate or bone flour in an attempt to obtain further information on the significance of the Ca/P intake ratio. The results obtained from this series of trials will be available in the near future.

SUMMARY

We have reported a series of metabolism studies in which the nitrogen, calcium and phosphorus balances of dairy cattle were determine for 12 ten-day periods, during which the daily milk yields varied from approximately 9 to 18 kgm. The rations fed consisted of alfalfa hay with and without supplements of starch, linseed oil meal, or di-sodium phosphate.

According to our data, cows in the early lactation period were usually in positive calcium balance; in negative nitrogen balance when the hay contained less than 2.0 per cent nitrogen; and always in negative phosphorus balance when only alfalfa hay was fed. Feeding di-sodium phosphate changed the negative phosphorus balances to slightly positive balances.

We have also included a general discussion of some of the factors which may be involved in rations restricted principally or solely to alfafa.

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THE CALCULATION OF ABSOLUTE VISCOSITY FROM DATA SECURED BY MEANS OF THE TORSION PENDULUM VISCOSIMETER*

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The Mojonnier-Doolittle viscosimeter is an instrument of the torsion pendulum type, designed for determining the viscosity of dairy products. This viscosimeter has found favor because of the ease and rapidity of operation, and simplicity of construction. It has the disadvantage, however, that the unit of measurement termed the degree of retardation, is relative rather than absolute. It has been impossible to compare data obtained with one of these instruments with readings obtained with another of this or any other type of viscosimeter. The authors have developed a method of converting degrees retardation, as measured by the Mojonnier-Doolittle viscosimeter into centipoise, the unit of absolute viscosity.

DESCRIPTION AND OPERATION OF THE TORSION PENDULUM VISCOSIMETER

The torsion pendulum was first used for the study of viscosity by Coulomb (1) in 1800. Babcock (2) in 1886 used a similar instrument for the study of the viscosity of milk, and Doolittle (3) in 1893 recommended that the instrument be used for the examination of oils. Babcock reported his viscosities in terms of standard sucrose solutions and this method was adopted by Doolittle (4).

More recently the instrument has been marketed by the Mojonnier Brothers Company under the name of the Mojonnier-Doolittle viscosimeter, and it has been widely used by workers in the dairy industry. That the present day "Mojonnier-Doolittle" instrument is still almost identical with the original

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viscosimeter used by Coulomb one hundred and thirty years ago may be seen from his drawing which is reproduced in figure 1.

A detailed description of the Mojonnier-Doolittle viscosimeter and its operation is given by Mojonnier and Troy (5). The general principle of the viscosimeter is the measurement of the resistance offered by a liquid to a torsion pendulum which is immersed in that liquid, the resistance or retardation effect being dependent upon the viscosity of the liquid. The sphere, which is

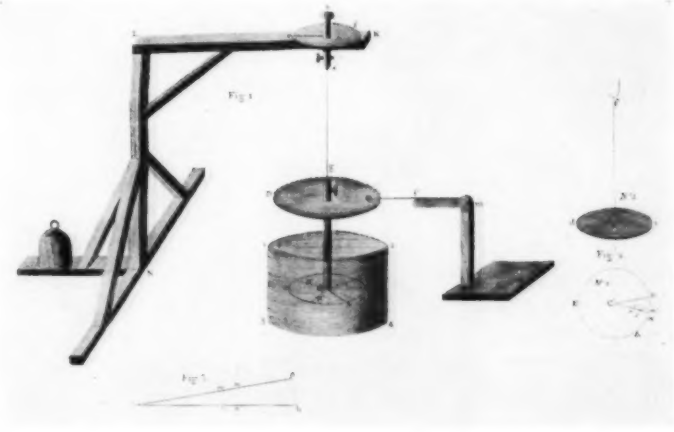

FIG. 1. THE ORIGINAL TORSION PENDULUM VISCOSIMETER DESCRIBED BY COULOMB IN THE YEAR 1800

suspended by a wire, is caused to oscillate by releasing it after it has been twisted through exactly one revolution. Upon being released, the sphere makes one complete and part of a second revolution, finally coming to a stop and then revolving in the opposite direction.

That part of a revolution by which the pendulum fails to ascend to its original degree of twist, when measured in degrees, is recorded as degrees retard. It may be read directly from a dial attached to the upper part of the pendulum.

DEVELOPMENT OF THE EQUATION

In order to discuss the motion of the viscosimeter ball, it will be convenient to designate certain things by symbols. Let us, therefore, represent the point of rest of the torsion pendulum as O, the starting point of the ball $-A$ and consequently the position which would be reached by the ball if there were no retarding force would be $+A$. The point actually reached by the ball on the first swing will be indicated as X, and the distance between X and A (called the retard) may be designated as R. Angular distances from the position of rest (O) may be designated by the letter S, or by a combination of symbols designating the points concerned. The viscosity of the material under investigation will be represented by V. The letter K with suitable subscripts will be used for various constants which will appear.

When the ball of the viscosimeter is rotated to position $-A$, a certain amount of potential energy will be stored in the wire. When the ball is released, this energy will be converted into kinetic energy as the ball passes from $-A$ to O, while from O to A the kinetic energy will again be transformed into potential energy. At the turning point the kinetic energy will have fallen to zero; and if no energy has been lost while the ball is in motion, the turning point will be at $+A$ where the potential energy is exactly equal to the potential energy at $-A$. In general, this will not be the case. Energy will have been lost from the system and the turning point will be at X instead of A. The difference between the potential energies of the positions A and X will be exactly equal to the energy lost from the sphere in passing from $-A$ to X. This difference in energy may be represented by the definite integral $K_1 \int_{OX}^{OA} SdS$ which on integration gives the difference in energy as equal to $K_2 (\overline{OA}^2 - \overline{OX}^2)$. It must be assumed throughout the discussion that the twist imparted to the wire at positions $-A$ and A is not great enough to alter the position of rest of the ball.

The energy lost from the torsion pendulum may, for convenience, be divided into two parts; the first part will be used up

in overcoming the internal resistance in the wire and the viscosity of the air; the amount of energy lost in this way will usually be small and may be considered as proportional to the square of the distance traversed by the pendulum ($\overline{OA} + \overline{OX}$). The second, and larger part will be lost in overcoming the viscous resistance of the material under investigation.

The energy required to move a surface through a viscous medium is proportional to the viscosity of the medium, the effective area of the surface, the distance traversed, the velocity of the surface with respect to the liquid and is an inverse function of the distance to the opposite wall. For a given viscosimeter ball centered in the same cup which is filled to the same depth each time, the surface area and the distance to the walls of the cup will be constant; therefore, the above reduces to $K_3 \cdot V \cdot$ velocity $\cdot$ distance. The distance traversed by the ball is ($\overline{OA} + \overline{OX}$). For small values of viscosity the pendulum will obey, approximately, the laws of harmonic motion and its mean velocity will be equal to $K_4 (\overline{OA} + \overline{OX})$. Substituting these values in the above equation, we find that the work done by the pendulum is $K_5 \cdot V \cdot (\overline{OA} + \overline{OX})^2 + K_6 (\overline{OA} + \overline{OX})^2$ where the last term represents the energy lost in the wire and air.

Equating the energy lost from the pendulum to the work done, we obtain:

$$K_2 (\overline{OA}^2 - \overline{OX}^2) = K_5 \cdot V \cdot (\overline{OA} + \overline{OX})^2 + K_6 (\overline{OA} + \overline{OX})^2$$

which reduces to

$$V = \frac{K(\overline{OA} - \overline{OX})}{(A + X)} - K_6$$

The equation may be simplified to the following form:

$$V = \frac{K \cdot R}{(2\,\overline{OA} - R)} - K'$$

DISCUSSION OF EQUATION

In the derivation of this equation, several assumptions have been made which limit its application. The errors introduced

by these assumptions should be understood before attempting to apply the equation.

It was first assumed that the energy lost in the internal resistance of the wire and the resistance of the air was proportional to the square of the amplitude of the swings. That this is justified was shown by the experiments of Coulomb (1), who states that he found "la diminution de l'amplitude à chaque oscillation resultante de cette imperfection (imperfect elasticity of the wire, and air resistance), etoit toujours proportionelle à l'amplitude des oscillations" (page 266).

A second assumption, and the most likely of all to be in error, is that the restraining force is proportional to the first power of the velocity. A full expression for the relation between the retarding force and the velocity would contain at least two terms; the first containing the velocity to the first power, and the second containing the velocity to the second power. For very low velocities, the second term may be neglected as was shown by Coulomb in the article cited; at higher velocities, the first term becomes negligible in comparison with the second term and many texts merely state that the retarding force is proportional to the square of the velocity. In the above equation, it is assumed that the velocity is low enough to safely neglect the second term. In the case of the Mojonnier-Doolittle instrument there may be some question as to the validity of this assumption, since the speed is much higher than was recommended by Coulomb and by Babcock. The error introduced in this way will be greater in the case of liquids of low viscosity and may entirely prevent the application of this equation to the instrument provided with a large viscosimeter ball.

Another assumption was made in deriving the equation: that the period of oscillation remains constant regardless of the viscosity. This is obviously not true but the deviation for the normal range of retard values is very small. It may be calculated and shown that for a retard of 120° the error is less than 1 per cent.

The assumptions regarding the constancy of the dimensions of

the instrument are obviously under the control of the operator and need no comment.

This equation has doubtless been used by other workers. The relations involved have been known for a long time, but the usual form of equation which is given is not applicable to the single observation recorded as degrees retard. In any case, the relation does not seem to be generally known to the users of the Mojonnier-Doolittle viscosimeter.

TABLE 1

Determination of constant K and calculation of absolute viscosity from degrees retardation of five standard oil samples

OIL SAMPLE NUMBER	(1) OBSERVED RETARD	(2) $\frac{R}{720-R}$	(3) KNOWN VISCOSITY	(4) K	(5) CALCULATED VISCOSITY	(6) ERROR	(7) ERROR
	degrees		*cp*		*cp*	*cp*	*per cent*
1	16	0.0227	19	1,806	19.61	+0.61	+3.21
2	28.5	0.0412	55	1,869	53.52	−1.48	−2.69
3	72	0.1111	186	1,872	181.6	−4.4	−2.36
4	112	0.1842	313	1,816	315.6	+2.6	+0.83
5	216	0.4285	751	1,803	763.4	+12.4	+1.65
				Mean 1,833			Mean deviation 2.14

THE VALIDITY OF THE EQUATION AND THE DETERMINATION OF THE CONSTANTS

In order to determine if the equation, based on the assumptions discussed above, is applicable to the Mojonnier-Doolittle viscosimeter, experimental work was performed. For this purpose five oils of known absolute viscosity were obtained. Table 1 gives the viscosity of these oils at a given temperature in centipoise, as determined by the Bureau of Standards; and the viscosity expressed in degrees retardation, as measured by the Mojonnier-Doolittle viscosimeter. In figure 2, the known values of viscosity were plotted against the experimental values of $\frac{R}{2A-R}$. By extrapolating to zero retard, the value of $-K'$ was found to be -22. This value of K' was then substituted in the original equation and then the data for each of the five oils were used to calculate the values of the constant K. These values are given

in column 4. The mean value of the constant K was then used to calculate backward and determine the apparent viscosity of each of the five samples. These values are tabulated in column 5, while the per cent error is given in column 7.

In using this equation, it is necessary to determine the value of the two constants by means of at least two liquids of known viscosity. The samples used in this work were petroleum oils standardized by the Bureau of Standards. For the instrument with a ball of approximately $1\frac{1}{4}$ inch diameter, samples with

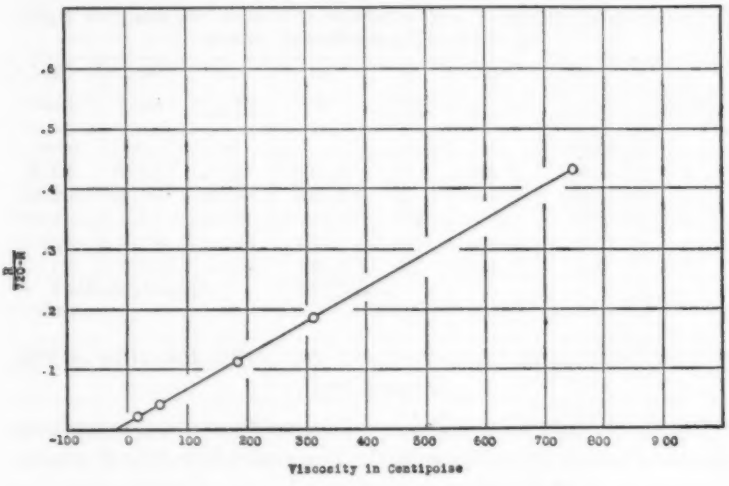

FIG. 2. GRAPHIC SOLUTION OF CONSTANT K

viscosities of approximately 50 and 500 centipoise respectively would probably be most satisfactory. The importance of close temperature control for accurate measurements cannot be too strongly emphasized.

SUMMARY

An equation has been developed which makes possible the conversion of degrees retardation, a measure of viscosity determined by means of the torsion pendulum (Mojonnier-Doolittle) viscosimeter, into centipoise, the unit of absolute viscosity.

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EFFECT OF HEAT (OESTRUM) ON BUTTERFAT PERCENTAGE AND MILK YIELD*

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Most breeders of dairy cattle believe that the period of heat (oestrum) appreciably affects both the milk yield and butterfat percentage. This belief is usually based on individual observation of a limited number of cows. Unfortunately the actual literature on the subject is very limited.

McClandish (1) states that there is a decrease in milk production on the day of breeding and the day following. His report also shows an apparently compensating increase in milk flow during the two days prior to the period of oestrum. Hooper and Brown (2) found that most cows showed a decrease in both milk and fat yields during the day of most evident heat. A few cows, however, exhibited an increase in production while others showed no variation.

DATA

The last 2025 Register of Merit records completed and accepted by the American Jersey Cattle Club were used in the following study. In the making of these records approximately 23,000 supervisions were conducted. Testers are supposed to indicate on each report if a cow is in heat during the period of supervision. We believe that this was usually done and the reports containing these notations were studied. Not all of the reports showing heat periods were used however. Only those one-day reports were selected which were preceded and followed by apparently normal supervisions. The butterfat percentage of the supervision made during the heat period was then compared with the average percentage of the preceding and succeeding supervisions. Similarly the milk yields of the one-day test period conducted

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while the cows were in heat were compared with an average of the milk yields for the two days preceding the test and the two days following. A total of 211 such comparisons were made. The results were tabulated and are given in the accompanying frequency table (table 1).

TABLE 1

Frequency table showing variations in fat percentage and milk yield due to "heat"

AMOUNT OF VARIATION IN FAT PERCENTAGE	NUMBER OF VARIATES SHOWING INCREASE	NUMBER OF VARIATES SHOWING DECREASE	AMOUNT OF VARIATION IN MILK YIELD	NUMBER OF VARIATES SHOWING INCREASE	NUMBER OF VARIATES SHOWING DECREASE
per cent			*pounds*		
0–0.10	16	15	0– 0.5	28	30
0.11–0.20	19	18	0.51– 1.00	18	23
0.21–0.30	22	10	1.01– 1.50	12	21
0.31–0.40	12	10	1.51– 2.00	3	15
0.41–0.50	11	8	2.01– 2.50	8	11
0.51–0.60	13	8	2.51– 3.00	1	5
0.61–0.70	10	6	3.01– 3.50	3	3
0.71–0.80	4	2	3.51– 4.00	3	
0.81–0.90	5	1	4.01– 4.50	2	4
0.91–1.00	1	3	4.51– 5.00		4
1.01–1.10	5		5.01– 5.50		3
1.11–1.20	1		5.51– 6.00	2	1
1.21–1.30	1		6.01– 6.50	1	1
1.31–1.40	1	1	6.51– 7.00		2
1.41–1.50	1	1	7.01– 7.50		2
1.51–1.60	2		7.51– 8.00		
1.61–1.70	1		8.01– 8.50		
1.71–1.80			8.51– 9.00		
1.81–1.90	1		9.01– 9.50		
1.91–2.00	1		9.51–10.00		
2.01–2.10			Over 10.00		2
2.11–2.20					
2.21–2.30					
2.31–2.40					
2.41–2.50					
Over 2.50		1			

An examination of the data showed that in 75 cases the period of heat resulted in an increased milk flow while in 131 cases the yield was lessened. The average variation in milk quantity during the twenty-four-hour test periods conducted while the cows were in heat compared with the average yield of the two

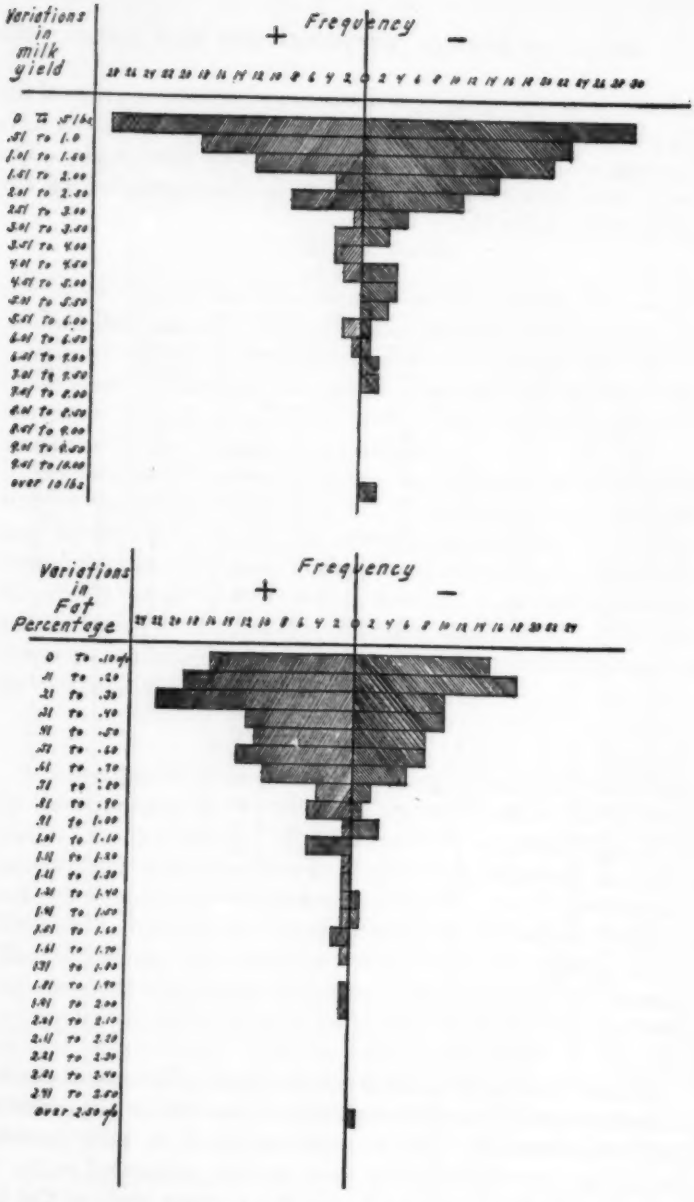

Fig. 1. Variations in the Milk Yield and Fat Percentage Due to "Heat"

preceding and two succeeding days was 1.67 pounds The standard deviation in milk quantity was 2.354 ±0.077. For 153 cows or 75 per cent of the total number, the variation in milk production was less than 2 pounds. The total of all tests gave an average decrease in milk yield of 0.63 pound.

Butterfat percentage exhibited a slight increase during 126 of the 211 supervisions made during heat and in the remaining 81 supervisions the percentage declined. The average variation in butterfat percentage was 0.43 per cent and the average increase for all supervisions was 0.13 per cent. Standard deviation for butterfat percentage was 0.449 ±0.015.

The frequency table grouping the variates of butterfat percentage and milk yield variations into certain classes, together with the accompanying charts graphically picture the variation in milk yield and fat percentage attributable to heat. Since both of the graphs are somewhat skewed, milk yield toward the minus (decrease) side and fat percentage toward the plus (increase) side, it was thought that a definite correlation might exist between the two. However, the coefficient of correlation between milk yield and fat percentage during heat was only −0.1002 ±0.003. In 43 cases both milk yield and fat percentage increased during the heat period and in 58 cases they both declined. There were 73 times in which the milk yield declined slightly and the fat percentage increased a small amount, and in 27 cases the reverse was true. In the remaining 10 cases either butterfat percentage or milk yield remained constant, showing no variation.

Another comparison was made which is not shown in the table. The milk yields of the two days preceding each heat period was compared to the yield of the two days following heat. For 112 cows the milk flow of the two days just prior to the heat period exceeded the yield of the two days after heat and in the other 99 cases the opposite was true. In 136 instances the difference in the yields of these two periods amounted to less than 3 pounds.

SUMMARY

From the data presented it would appear that:

1. The effect of heat (oestrum) on milk yield and butterfat percentage is quite limited.

2. The slight difference exhibited seems with most cows to be toward a small decline in milk flow and an equally limited increase in butterfat percentage during the period of heat. Individual cows may show variation in the opposite direction or no variation at all.

3. No compensating increase could be noted in the milk production just prior to the heat period.

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THE USE OF THE MOJONNIER MILK TESTER FOR THE ROUTINE DETERMINATION OF VANILLIN WITH PRELIMINARY DISCUSSION OF THE TOWT LEAD NUMBER*

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Most flavoring materials used in vanilla ice cream at the present time are either concentrated vanilla preparations or extracts which have been reinforced with vanillin. The choice of such materials over the straight vanilla extract has undoubtedly been brought about by the attempt of the ice cream manufacturer to prevent "freezing out," the desire of the consuming public for a stronger flavored ice cream, the advent of high solids ice cream which necessitates more flavor, and the regulations in certain states which prohibit the use of coumarin and other foreign flavoring materials.

Consequently, because vanillin is the most expensive ingredient of the extract and because it plays such an important part in the quality of the ice cream produced, its routine determination affords one of the most important means of checking the quality of the product purchased. Today most ice cream manufacturers buy in large quantities and contract for a year's supply in advance. They must feel assured that at all times during the period of the contract they are getting exactly what was originally specified.

For those dairy laboratories where both the Mojonnier and Babcock testers are found the following method affords a simple and very accurate means of determining the vanillin content and estimating the bean content of an extract.

PROCEDURE

Pipette 25 cc. of the extract into a petri dish cover and add 10 cc. of water. Evaporate to about 25 cc. under the suction fan

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on the hot plate of the Mojonnier Tester. The temperature of the fat hot plate may be kept at 60°C. during this process but care must be taken that the temperature does not rise above 60°C. Add 10 cc. water and evaporate again to about 25 cc.

Transfer the dealcoholized extract to an 18-gram cream test bottle which has previously been calibrated to 50 cc. To calibrate add exactly 50 cc. of distilled water at 20°C. and mark on the neck of the bottle. The transfer to the cream test bottle from the petri dish cover can be made very easily by using a glass funnel and stirring rod. Wash the petri dish cover clean using a wash bottle containing warm water.

Add 4 drops of Glacial Acetic acid and shake thoroughly. Add 10 cc. of standard lead acetate solution (80 grams C. P. crystallized lead acetate made up to 1 liter) make up to the 50 cc. mark with water and shake thoroughly. Cork the cream test bottle and centrifuge in a Babcock Centrifuge for five minutes at same speed used in making a fat test. Pipette off 25 cc. of the supernatant liquid, which should be clear, into a Mojonnier fat extraction flask and reserve the remainder for the estimation of the volume of lead acetate precipitate.

Extract the vanillin solution with three 20-cc. portions of ether which has previously been washed twice with distilled water, using the Mojonnier centrifuge after each shaking at the same speed and time as used in the fat test. Pour the ether extractions into a weighed solids dish and after the last extraction lower the dividing line by pipetting off the lower liquid. The same care used in pouring off the ether solution in the fat test should be used in this test.

Evaporate the ether solution of vanillin on the fat hot plate with the temperature not above 55°C. dry over sulphuric acid to constant weight and calculate the vanillin as grams per 100 cc. or as per cent by weight if the specific gravity of the original sample is known.

To check the accuracy of this method of determining vanillin different amounts of vanillin were added to an extract of known vanillin content and analyzed. The results are given in table 1.

The accuracy of this method even in samples containing large

amounts of added vanillin is apparent from the table. In the official Hess and Prescott method, of which this is a modification, it is claimed that the vanillin extracted is never pure but contains varying amounts of gummy resinous matter; such is probably the case in this method, but apparently the error due to impurities just about offsets the loss of incomplete extraction.

TABLE 1

Accuracy of results secured by the proposed method of estimating vanillin

VANILLIN PRESENT IN EXTRACT	VANILLIN ADDED	TOTAL	FOUND	TOTAL RECOVERED	REMARKS
per cent	*per cent*	*per cent*	*per cent*	*per cent*	
0.40	0.2051	0.6051	0.635	105.0	Bourbon extract
0.40	0.8022	1.202	1.20	99.7	Bourbon extract
0.245	0.4797	0.7247	0.706	97.7	Tahiti extract
	2.02	2.02	2.02	100.0	Vanillin dissolved in 38 per cent alcohol
	0.491	0.491	0.53	108.0	Vanillin dissolved in 38 per cent alcohol
	1.43	1.43	1.42	99.3	Vanillin dissolved in 38 per cent alcohol
0.245	1.527	1.77	1.69	95.5	Tahiti extract
0.245	0.4797	0.7247	0.715	98.7	Tahiti extract
	0.6049	0.6049	0.582	96.4	Aqueous vanillin solution
0.245	0.4797	0.724	0.756	104.3	Tahiti extract
	1.381	1.381	1.396	101.0	Aqueous vanillin solution
	0.138	0.138	0.134	97.1	Aqueous vanillin solution
0.233	1.40	1.63	1.67	102.4	Bourbon and Tahiti 50–50, simple syrup added
0.294		0.294	0.294	100.0	Tahiti extract
0.147		0.147	0.150	102.0	Same as above diluted 1:1
Average.....				100.5	

The determination of vanillin and the estimation of volume of lead acetate may be made easily in one day with this method, while the official methods would require at least three days. This method is also well adapted for volume as at least eight samples may be run at one time, and much more cleaner extraction can be made with the Mojonnier Flask than with a separatory funnel. The colorimetric method for determination of vanillin is shorter

but involves the use of a colorimeter which is an instrument rarely found in a dairy laboratory and it does not allow for a lead number or bean matter estimation of the same weighed sample.

To determine the volume of lead acetate precipitate make up the remaining solution in the cream test bottle to the 45 per cent mark with water, cork, invert, and shake thoroughly, taking care that no particles of bean matter adhere to the bulb of the bottle. Centrifuge in the Babcock Centrifuge with the neck and corked end out at 1000 r.p.m. for five minutes. This is accomplished by inserting a round tube of wood about 6 inches long with a $\frac{5}{8}$-inch diameter hole in the center in the bottle carrier on the centrifuge and placing the bottle neck in the hole in this cylinder of wood. This forces the lead acetate precipitate into the graduated neck where it can be measured with the dividers and read on the graduated scale.

A comparison of readings obtained in this manner was made with the normal lead number on six different samples with the following results:

The centrifuge used was a 36-bottle, 22-inch, steam driven, Babcock tester and was run at 1000 r.p.m. for five minutes.

SAMPLE NUMBER	LEAD NUMBER	READING ON 18-GRAM CREAM TEST BOTTLE
1	0.24	4.5
2	0.49	21.5
3	0.86	30.0
4	0.29	8.0
5	0.57	23.5
6	0.43	17.5

This simple test affords a very rapid method of checking the consistency of a product. It is particularly desirable when one buys on a "key sample" basis. In this case the seller submits a large sample of the flavoring material decided upon with the understanding that each shipment during the period of the contract shall conform to the composition of the key sample. After having carefully determined the lead reading of the key sample any variation of subsequent shipments can easily be detected without resorting to the tedious process of determining the official lead number.

A COMPARISON OF THE MODIFIED BABCOCK AND THE MOJONNIER METHODS FOR BUTTERFAT IN ICE CREAM*

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The object of this paper is to give the results of a comparison of the modified Babcock and the Mojonnier methods for butterfat in Ice Cream under factory conditions. The methods used in this paper were strictly factory methods used for standardization of the ice cream mix or for a check on this standardization. The Mojonnier method was used for standardization and then a Babcock test run on the same sample. The methods used are as follows:

MODIFIED BABCOCK

A 9-gram sample of the ice cream mix was weighed into the California standard 50 per cent 9-gram test bottle, using the standard Babcock torsion balance. Fifteen cubic centimeters of glacial acetic acid were then added to the bottle and agitated until all lumps were broken up. The acid used was commercial glacial acetic acid (98 to 99 per cent) and was kept in a tightly corked bottle. The sample was heated in a water bath to approximately 110°F. While still hot small portions of sulphuric acid were added and mixed with the sample. This was repeated until the sample developed a dark brown color (almost black) within two minutes standing. This is the most important part of the procedure and considerable experience was necessary to judge the proper color. The action was stopped by the addition of a few cubic centimeters of water and the sample centrifuged as usual. The standard five-, two- and one-minute centrifugings are satisfactory although a longer time is desirable on the last two. The samples were brought to 135°F. in a water bath and the fat read carefully (using glymol oil) with fine point dividers.

* Received for publication May 20, 1929.

The effect of further centrifuging was found to be very slight providing it was done properly in the first place. A 14-inch Burrell steam turbine was used at a speed of 900 to 1000 revolutions per minute. In a few cases a third washing and rapid centrifuging lowered the test about 0.05 per cent. The fat in this case was almost transparent.

An experienced operator can almost always obtain a perfect test with this procedure properly carried out. A test was considered perfect only if the fat column was a golden yellow, almost transparent, and homogeneous throughout. No burnt, charred or unburned substance was allowed in the fat column or in the liquid immediately below the column. Such a test shows improper manipulation and should be repeated. As a general rule an experienced operator can get a better looking fat column with this test than with the standard Babcock test for milk or cream.

The bottles used should be very carefully selected for accurate graduations. The graduations are to 0.5 per cent and are read to 0.10 per cent by judging tenths between the graduations. Duplicate tests showed a maximum variation of 0.1 per cent. An occasional 0.2 per cent or over was recorded but on further testing proved to be due either to faulty testing or a faulty bottle. The usual variation was well within the accuracy of reading, that is ± 0.05 per cent.

It should be said, however, that this test is only applicable to ice cream that contains no fibrous flavoring material. Experiments show that strawberry, chocolate, and such flavors can not be tested satisfactorily by this method.

The Babcock-Gerber method as proposed by Moore and Morse[1] was tried in comparison with the modified Babcock and the Mojonnier. The results were not good, as all tests recorded were invariably from 0.2 to 0.4 per cent higher than either the Babcock or the Mojonnier. The amount of centrifuging affected the results considerably and the fat always carried the odor of butyl alcohol. The best results the authors gave for their method do not seem comparable to those we obtained. The only advan-

[1] Moore and Morse: Jour. Dairy Sci., 1926, ix, 276–285.

tage noted was the ability to get a clear fat column with chocolate and strawberry ice creams but even here the results did not check with the Mojonnier figures.

MOJONNIER METHOD

The standard Mojonnier method for butterfat in ice cream was used.

TABLE 1

Comparison of Babcock and Mojonnier tests

BABCOCK TEST (DUPLICATES)		MOJONNIER TEST	DIFFERENCE, BABCOCK FROM MOJONNIER
per cent	per cent	per cent	per cent
11.1	11.1	10.94	+0.11
12.2	12.2	12.01	+0.19
11.0	11.0	10.94	+0.06
14.05	14.1	14.13	−0.05
11.20	11.20	11.15	+0.05
11.20	11.10	10.98	+0.17
11.0	11.10	10.96	−0.09
12.0	12.0	11.94	+0.06
12.0	12.0	12.00	+0.00
11.05	11.0	11.05	−0.03
11.2	11.1	11.14	+0.01
11.2	11.2	11.11	+0.09
18.1	18.1	18.00	+0.10
14.05	14.05	14.09	−0.04
18.5	18.6	17.96	+0.59
15.85	15.90	15.75	+0.12
17.20	17.20	17.11	+0.09
11.00	11.00	11.05	−0.05
13.94	14.00	13.95	−0.02
12.20	12.15	12.20	−0.03
Average 0.03 difference between duplicates		Average 0.10 difference from Mojonnier	

The fat determinations were all made at maximum speed, as they were used for factory standardization. The minimum time required for a fat and solids test was 40 minutes for 1 test, and 1 hour and 25 minutes for 4. The accuracy under these conditions was within ±0.05 per cent on duplicate samples. Curved five gram pipettes were used to weigh in the samples. In case of the

slightest amount of rendered fat the sample was weighed directly into the bottle. Two extractions were used, a third not proving necessary.

RESULTS

The first three tables show the relative accuracy of the two methods and also the error on duplicate samples by the same

TABLE 2

Duplicate Mojonnier tests on separate samples

SAMPLE NUMBER	MOJONNIER TESTS ON SEPARATE SAMPLES		DIFFERENCE
	per cent	*per cent*	*per cent*
1	11.26	11.29	0.03
2	13.80	13.78	0.02
3	14.45	14.40	0.05
4	11.13	11.13	0.00
5	15.89	15.92	0.03
6	14.43	14.43	0.00
7	11.41	11.46	0.05
8	11.04	10.93	0.11
9	14.44	14.37	0.07
10	12.63	12.59	0.04
11	11.35	11.35	0.00
12	11.13	11.08	0.05
13	11.15	11.15	0.00
14	17.51	17.55	0.04
15	11.96	12.01	0.05
16	11.10	11.12	0.02
17	10.92	10.91	0.01
18	11.06	11.02	0.04
19	11.14	11.14	0.00
20	16.02	15.98	0.04

Average variation 0.03 per cent

method. Table 1 contains 20 Babcock and table 2 contains 20 Mojonnier duplicate determinations. Table 3 shows tests of 10 samples on which duplicates were run by both methods.

Seven hundred and fifty-four tests were recorded. The Mojonnier test was in all cases used as the standard. The Babcock tests are divided into three classes; those that yield higher values, those that check exactly, and those that yield lower

values than the Mojonnier method. The results are considered to 0.01 per cent butterfat.

273 tests were lower than Mojonnier
31 tests were the same as Mojonnier
450 tests were higher than Mojonnier

TABLE 3

Comparison of duplicate tests by both methods

SAMPLE NUMBER	BABCOCK TEST		MOJONNIER TEST		DIFFERENCE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	17.70	17.70	17.78	17.78	−0.08
2	13.70	13.65	13.56	13.63	+0.08
3	10.50	10.50	10.28	10.28	+0.22
4	10.70	10.65	10.59	10.59	+0.08
5	10.60	10.60	10.59	10.61	−0.00
6	10.50	10.50	10.36	10.42	+0.11
7	10.70	10.70	10.71	10.69	0.00
8	12.00	12.00	11.97	11.95	+0.04
9	10.50	10.45	10.49	10.41	+0.03
10	12.00	12.00	11.98	12.01	0.00

TABLE 4

Variation of Babcock and Mojonnier tests

GROUP NUMBER	NUMBER OF TESTS	PERCENTAGE OF TOTAL TESTS	RANGE OF VARIATION FROM STANDARD MOJONNIER TESTS
			per cent
1	5	0.6	±0.5 to 0.6
2	8	1.1	±0.4 to 0.5
3	21	2.8	±0.3 to 0.4
4	72	9.5	±0.2 to 0.3
5	204	28.8	±0.1 to 0.2
6	444	59.2	±0.0 to 0.1
Total.....	754	100.0	

The tests are grouped as to their variations from the Mojonnier test in table 4. An average of all the tests shows that the Babcock method gave results which are 0.04 per cent higher than the Mojonnier method. A further check of the data shows 45.2 per cent of the tests check within ±0.06 per cent, that is within Mojonnier accuracy.

All samples in groups one to three inclusive were retested to check any possible errors in testing. Any mistakes found were further rectified by a third test.

DISCUSSION

This paper to the best of our knowledge represents a more comprehensive study than has heretofore been published on the relative accuracy of the Mojonnier and Modified Babcock Methods when applied to ice cream. It represents tests, all of which were made under strictly factory conditions. About 85 per cent of the Babcock tests were made by one man and about 90 per cent of the Mojonnier tests by another operator. The other tests were interchanged between the two men. Different operators were used on both tests to remove the personal element as much as possible. No appreciable difference could be noted with either operator on either test.

Eighty-six per cent of the tests were satisfactory if we consider ± 0.2 per cent as sufficient accuracy. The other 14 per cent are of doubtful use in standardization. No explanation is offered for these tests nor is any remedy proposed. A correction of 0.04 per cent subtracted from all Babcock tests would make a greater number of tests check with the Mojonnier method. The reason for this is that there are 450 tests higher and only 270 tests lower.

An attempt to analyze the fat column of a Babcock test by the Mojonnier method gave no conclusive results. Determinations on half a dozen samples showed that the fat is never 100 per cent pure. The results did not show consistently that the fat column on a sample testing high by the Babcock method, actually contained a smaller percentage of ether soluble material than those samples in which the agreement was better although there was some indication of it. The fat columns obtained in the Babcock determinations tested from 97.53 to 98.7 per cent fat and the balance was moisture. This was not checked very thoroughly but it is logical to believe that a sample that tests high by the Babcock method would yield a fat column which contains a lower percentage of fat. A low test could be explained by incomplete separation of the fat from the acid liquid.

The question has been raised whether it is justifiable to read a cream test bottle to 0.1 per cent. The theory is that it is false accuracy to read closer than the smallest division on the graduated scale. We have assumed nevertheless that it is possible, and our results have confirmed it. However, this is only possible by using the very best equipment and the most careful technique. It must be said to the credit of our glassware manufacturers that the test bottles supplied are very accurate, to a much greater degree of precision than is required by law.

Another question is of the relative merit of using a 9-gram sample in a milk bottle and doubling the result, as against this method. According to theory this should be the best as no "false accuracy" is involved in reading to 0.2 per cent. However, on doubling a test a meniscus error is introduced which might offset any greater accuracy in reading. By comparison in this laboratory the milk bottle invariably showed from 0.2 to 0.6 per cent higher than the corresponding cream bottle test, which test has been shown to average slightly higher than the Mojonnier. The conclusion was that the cream bottle test using glymol to remove the meniscus gave the most consistent results. However, there are many other small variations in this test, such as the strength of the acetic acid used, strength of the sulphuric used and relative amounts of these reagents, that were only worked out so as to give satisfactory tests without regard to their effect, if any, on the test. There is still room for much research on these and many other problems of this test.

CONCLUSIONS

Forty-five per cent of the modified Babcock tests checked with the Mojonnier and 86 per cent were satisfactory, that is within ± 0.2 per cent of the Mojonnier test. No reason can be given as to why some samples do not check within ± 0.2 per cent. The maximum variation was approximately 0.5 per cent. The Babcock tests averaged 0.04 per cent higher than the Mojonnier tests.

ACKNOWLEDGMENT

We are indebted to the Division of Dairy Industry of the University of California, particularly to Dr. C. L. Roadhouse and D. H. Nelson, whose comments and criticisms on this paper are gratefully acknowledged.

VARIATIONS IN THE MILK FROM DIFFERENT QUARTERS OF THE SAME UDDER: THEIR SIGNIFICANCE IN STUDIES OF COAGULABILITY*

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The study of the factors determining the coagulability of milk by heat is complicated by a number of possibly interacting variables difficult to control. Moreover, as has been indicated elsewhere[1] each sample is an individual system, so that milk from one normal cow can not be used as a check on that from another, neither can a sample from one milking be used as a control on a previous or subsequent milking from the same cow. Variations in the thermostability, the alcohol test, and the hydrogen-ion concentration of herd milk have been attributed, and may be due, to changes in feed or environmental conditions. It is well known that milk at the beginning or end of lactation is unstable and there is a good deal of evidence that subacute infectious processes in the udder may cause alterations in the balance of ingredients without noticeably reducing the volume of milk secreted.

In studying the day to day variations in the milk of individual cows, it was noticed that not only does the milk of a single cow vary from one milking to another but that it varies even from different quarters of the udder. It was found that if quarters are milked separately the samples may be no more alike than if they had been obtained from different cows. The four quarters of the udder, therefore, are separate units physiologically as they are anatomically. Furthermore these variations are not constant—samples from each quarter vary from day to day just as does the total pooled sample. The data in table 1, obtained from six morning milkings over a period of two weeks, are illustrative.

* Received for publication May 22, 1929.

[1] Benton, A. G., and Albery, H. G.: Jour. Biol. Chem., lxviii, 251 (1926).

The hydrogen-ion concentrations were determined electrometrically. The coagulation times were determined after the milk had been forewarmed and condensed to 18 per cent total solids.

TABLE 1

Differences in milk from different quarters of the udder of cow 258 of the Beltsville herd

DATE	QUARTER OF UDDER	VOLUME	FAT	SERUM SOLIDS	pH	ALCOHOL TEST	COAGULATION AT 120°C.	BACTERIAL COUNT
		cc.	*per cent*	*per cent*			*minutes*	
July 20	Left rear		1.7	8.52	6.65	−	9	None taken
	Left fore		2.0	7.9	6.78	−	15	None taken
	Right fore		2.1	7.76	6.61	+	7	None taken
	Right rear		2.0	8.1	6.62	−	13	None taken
July 22	Left rear	1,700	3.0	8.52	6.62	−	9	19,000
	Left fore	1,460	3.0	8.55	6.63	−	8	16,900
	Right fore	1,150	3.0	8.56	6.62	−	6	12,500
	Right rear	2,000	3.0	8.59	6.62	−	12	15,100
July 24	Left rear	1,770	2.5	9.23	6.55	−	19	2,000
	Left fore	1,140	2.1	8.01	6.66	−	17	69,000
	Right fore	1,600	2.3	8.45	6.58	−	8	1,900
	Right rear	1,960	2.4	8.71	6.65	−	5	1,300
July 27	Left rear	1,470	2.9	8.7	6.67	+	3	14,300
	Left fore	1,030	2.3	8.34	6.77	−	5	10,200
	Right fore	1,445	2.7	8.60	6.67	+	0*	4,700
	Right rear	1,540	3.0	8.72	6.65	+	4	12,800
July 29	Left rear	1,575	2.6	8.84	6.66	+	5	None taken
	Left fore	1,000	2.5	8.31	6.84	−	12	None taken
	Right fore	1,455	2.4	8.5	6.70	+	3	None taken
	Right rear	1,555	2.5	8.73	6.63	+	5	None taken
August 3	Left rear	1,630	2.9	8.46	6.66	−	10	None taken
	Left fore	1,120	3.2	8.14	6.75	−	16	None taken
	Right fore	1,490	3.3	8.41	6.65	+	6	None taken
	Right rear	1,580	3.0	8.3	6.65	+	8	None taken

* Coagulated before it reached 120°C.

Variations similar to these in kind and degree were observed in a number of cows in the Beltsville herd of the Bureau of Dairy Industry, indicating that such cows are fairly common. Indeed,

it may be the general rule rather than the exceptional state of affairs. In the work done so far, alcohol tests were made in the stable at milking time and cows which gave alcohol positive milk from one quarter and negative from another were chosen for further study. It should be noted that in the case here presented all four samples were alcohol negative on July 24, and yet they were alike in no other particular.

Such variations when encountered in a cow giving several liters at a milking provide an excellent opportunity to study certain factors affecting stability, since obviously other variables such as age of cow, stage of lactation, change of feed, digestive peculiarities or upsets, weather, sunlight, and exercise are completely ruled out. All four samples are elaborated simultaneously from an identical blood supply, and the difference in ingredients, hydrogen-ion concentration and coagulability must be due to differences in the secreting mammary epithelium or to chemical changes that take place while the milk is still in the udder. Studies of the variations in type and numbers of the udder bacteria should yield significant results. In the observations made thus far high counts were not so apt to accompany loss of stability as to precede it. This would point to the effect of the bacteria on the secreting cells rather than on the milk itself, but not enough work has been done to justify any definite opinion.

As the writer is no longer able to continue this work these suggestions are offered to other investigators for what they may be worth.

SEASONAL VARIATIONS IN THE FREEZING POINT OF MILK*

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It has been reported by a large number of workers that the freezing point of milk was quite definite. On reflection that is quite a reasonable assumption because the compounds of milk, sugar and ash, which would exert the greatest effect in lowering the freezing point, are the ones that are known to be the least variable in milk. An examination of the work of a large number of investigators in this field will show that there is an agreement with regards to the fact that the freezing point of normal milk falls within rather narrow limits. The highest freezing point, −0.540°C., is reported by Gooren (1) and the lowest limit, −0.575°C., by Ducrose and Imbert (2). Our investigations show the extreme limits to be between −0.537 and −0.582°C. (table 1).

The effect of the season on the depression of the freezing point has received very little attention. Stoecklin (3) after examining 2500 samples makes the following generalization: milk, freshly drawn, from any cow, whatever the breed, from whatever region of the country, from animals in stable or in pasture, whether poorly or substantially fed, whether drawn from a period near or remote from parturition, in winter or summer, morning or evening, whether yield be scant or abundant, has a definite freezing point which varies but little from −0.550°C. although under various influences the chemical composition changes in enormous proportions. Hortvet in his very extended work on the cryoscopic index of milk (4) seems not to have noticed the seasonal variation. However, the authors (5) noticed the effect of the season on the depression of the freezing point but sufficient data was lacking at that time. Since then a larger amount of data have been collected. All determinations were made on mixed,

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unpasteurized, herd milk using the Hortvet Cryoscope. The freezing was carried out immediately after obtaining the sample.

EXPERIMENTAL DATA

Table 1 for the most part is self-explanatory except the wide variations in the same month, and even from day to day, are

TABLE 1

Distribution of freezing points of herd milk (by months, 1924–1927 inclusive)

(Figures in months column indicate number of determinations)

FREEZING POINT DEPRESSION	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	TOTAL
°C.													
0.5800–0.5824		1				2							3
0.5775–0.5799	2						1	2					5
0.5750–0.5774		1											1
0.5725–0.5749													0
0.5700–0.5724		1					1	1				1	4
0.5675–0.5699	3	1	1			2	2	2				2	13
0.5650–0.5674	2	1	1	1				1			1		7
0.5625–0.5649		3		1		1	3	3		1	1		13
0.5600–0.5624		2	1	3	5	2	3	4		1			21
0.5575–0.5599	2	1	2	2	2	2	3	1			1		16
0.5550–0.5574			1	1	3	4	4	2		3			18
0.5525–0.5549			1	2	1	4	3						11
0.5500–0.5524			1	1	2	3	1				1		9
0.5475–0.5499			2		2	1							5
0.5450–0.5474				1									1
0.5425–0.5449					3	1							4
0.5400–0.5424				1									1
0.5375–0.5399													0
0.5350–0.5374										1			1

difficult of explanation. Probably an explanation on meteorological grounds holds possibilities. Jackson and Rothera (6) were able to show some correlation between weather and conductivity of milk serum. On hot, dry days the conductivity was high while on cold days with high humidity the conductivity was low.

Their analyses for lactose content showed higher values when the conductivity was low and vice versa. However, our attempt at correlation of meteorological data and the wide variations was not very successful.

The data for October, November and December (1927) should be mentioned as it was collected during one season only. This season was rather exceptional as July, August and first part of September were very dry resulting in very poor pasturage conditions. This was followed by much rain and warm weather practically duplicating pasturage conditions found in early spring.

TABLE 2

Average freezing point depression (by months and years)

MONTHS	1925	1926	1927	GENERAL AVERAGES
January	−0.5673		−0.5627	−0.5650
February	−0.5672		−0.5622	−0.5647
March	−0.5636	−0.5587	−0.5517	−0.5580
April	−0.5572	−0.5548	−0.5581	−0.5567
May	−0.5566	−0.5536	−0.5577	−0.5559
June	−0.5556	−0.5645	−0.5593	−0.5598
July	−0.5567	−0.5633	−0.5561	−0.5587
August	−0.5604	−0.5626	−0.5635	−0.5622
September				
October			−0.5549	
November			−0.5594	
December			−0.5602	

The data for the other months represent normal conditions found in this climate.

Attention is called to the fact that in the last column of this table, the greater number of depressions fall between the limits of −0.5475 and 0.5724°C.

Table 2 was constructed from the data shown in table 1 representing the freezing point depressions between the limits of −0.5724 and −0.5475°C. defined by the heavy horizontal lines. It was thought that the other data are too much scattered and would greatly magnify the averages. The data in this table represents an average of the freezing point depressions per month

during the years over which the investigation was conducted. The last column represents an average by months during the entire period.

The curves in figure 1 were constructed from the data in table 2.

Curve A (1925). This curve, as well as B and C, was made from the average freezing point depressions from the milk of a mixed herd. During the months of January and February the

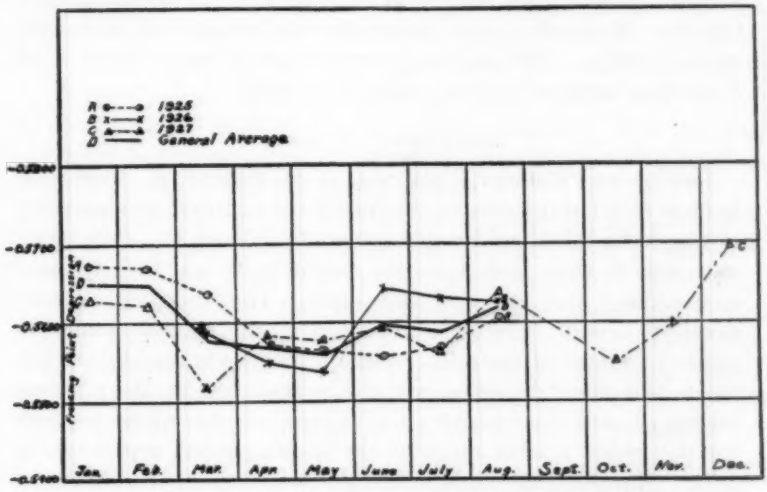

FIG. 1. EFFECT OF SEASON ON DEPRESSION OF FREEZING POINT

herd received the usual winter rations of silage and rough dry feed. However, in March a small amount of pasturage became available, with a luxuriant growth in April, May, and June with a waning in July to a scant growth in August due to the hot, dry weather.

Curve B (1926). Normal spring pasturage conditions existed for the spring months (March, April, and May). Exceptional drouth conditions prevailed during June, July, and August, resulting in very scant pasturage especially during June. A consequent lowering of the freezing point depressions resulted.

Curve C (1927). Unusual pasturage conditions existed during March. Average conditions prevailed during remainder of the spring months with the usual drouth conditions during August. However, warm September rains resulted in a practical duplication of spring pasturage with an attendant fluctuation of the freezing point depression.

Curve D (general average). This curve was constructed from a general average of the freezing point depressions of the other curves. It is carried only to September 1 as the work through October, November, and December was conducted only one season (1927). The pasturage conditions during October and November were exceptional rather than usual.

DISCUSSION OF DATA

Jackson and Rothera, University of Melbourne (6), were able to show that hot dry weather increased the electrical conductivity of raw milk while cold wet weather diminished it. Also, they were able to show that when the conductivity was high the lactose content was low and vice versa. The variation in conductivity would indicate a variation in the soluble, ionizable mineral content of the milk. Since a balance is maintained between this mineral content and the lactose content, the greatest factors causing the freezing point depression, this would account for the rather narrow range of the freezing point depression of milk. However, since this is thought to be dependent on meteorological conditions it may be one of the principal factors in the wide variations from day to day but it does not account satisfactorily for the general seasonal variation.

Cranfield, Griffiths, and Ling (7) were able to show a considerable variation in soluble ash content of milk with the months of the year, the percentage being much higher in summer than in winter. Sodium chloride is conceded to be the principal constituent in the soluble ash of milk and one of the most variable factors, hence it is reasonable to assume that it is the factor used to maintain the osmotic equilibrium between the blood and milk secretion in the animal.

Woodman, Blunt, and Stewart (8) were able to show that the

mineral content of pasturage, especially the calcium content, showed a very definite seasonal variation, increasing to a maximum in the dry mid-season and later falling off again. This increased calcium content if transferred to the milk would not greatly affect the freezing point depresssion as possibly two-thirds of the total phosphate is in suspension as $Ca_3(PO_4)_2$. These investigators have shown that the calcium content varies from season to season (9). They are inclined to believe that meteorological conditions produce these variations. Fagan (10) has been able to show that when grasses, cut or grazed very short, are much more leafy when they grow out again and consequently the chemical composition changes greatly. This is undoubtedly what happens when the Autumn rains come on. However, this worker has shown that this type of growth is much poorer in nutritive value than new spring growth.

Undoubtedly, when cattle are first placed on new pasture in the spring a physiological disturbance results due to the nutritive content of the grass and meteorological changes. This brings on a disturbance of the osmotic equilibrium maintained in the body and consequently a variation in the freezing depression of milk results. Curve C, figure 1, would justify these conclusions since practical spring pasturage conditions were duplicated in October and hence the raising of the freezing point depression.

SUMMARY

1. There is a distinct seasonal variation in the freezing point depression of milk; the greater depression being in the spring months, the lower portion during the winter months.

2. Change in meteorological conditions offer an explanation of the wide variations of the freezing points encountered from day to day.

3. The seasonal variation of the freezing point depression is due to a change in the osmotic equilibrium maintained in the body of the animal. While meteorological changes may be a factor, the change in chemical content of the animal's food during the season is the controlling factor in the seasonal variation of the freezing point.

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THE OVARIAN HORMONE CONTENT OF PREGNANT COW'S URINE*

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INTRODUCTION

Because of the close correlation between the mammary glands and the reproductive organs, the pioneer investigations of Allen and Doisy (2, 3, 4, 6, 9), Frank and associates (12, 13, 14), Parkes and Bellerby (24) and others, concerned with an ovarian hormone, are of especial interest for they have furnished fundamental knowledge concerning the hormone which is believed to activate or stimulate the growth of the mammary gland.

That the ovaries are the organs which cause the phenomenon of "heat" in females has long been known. It was shown by Knauer (18) that this phenomenon could be abolished when both ovaries were removed.

Similarly it was found by Halban (15) that by the removal of the ovaries from guinea pigs the uterus and mammary gland ceased to develop; however, when ovarian grafts were made, the generative organs developed normally.

Although the early work proved that the ovaries controlled the functions of the generative organs, a method was lacking by which specific substances from the ovaries could be isolated and tested.

The pioneer investigations leading to a definite test of the presence and potency of the oestrous producing hormone was made by Papanicolaou and Stockard (22) who upon microscopical examination of the vaginal contents of the guinea pig showed that there were distinct changes in the cellular content of the uterus and vagina during the oestrous cycle.

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Later the oestrous cycle was studied by means of the vaginal smear technique in the rat by Long and Evans (21) and in the mouse by Allen (1).

The early work of Halban (15) and of Knauer (18) proved rather conclusively that a secretion from the ovaries carried by the blood stream served to control the sexual functions. Therefore, it could be supposed that ovarian control was probably due to some hormone elaborated by the ovaries. The first to show that there was a specific hormone secreted by the ovaries were Allen and Doisy (3) who produced typical oestrous changes, as

TABLE 1

Source of an ovarian hormone as tested for by vaginal smear technique

SOURCE	INVESTIGATOR
Ovaries (sheep)	Allen and Doisy (1923)
Liquor folliculi	Allen and Doisy (1923)
Corpus luteum (Human)	Allen, Pratt, and Doisy (1925)
Wall of corpus luteum	Zondek and Ascheim (1925) (26)
Placenta	Allen and Doisy (1924)
Maternal placenta	Doisy, Ralls, Allen, and Johnston (1924)
Fetal placenta	Parkes (1928)
Amniotic fluid	Parkes (1928)
Blood of females (during oestrous cycle)	Frank, R. T., Frank, M. L., Gustavson, R. G., and Weyerts, W. W. (1925)
Blood of pregnant females	Fels (1926)
Urine (during oestrous cycle)	Loewe (1926) (20)
Urine (during pregnancy)	Veler and Doisy (1928)

shown by vaginal smears, in spayed animals, by the injection of liquor folliculi from hog ovaries. The discovery of the oestrous producing hormone in the follicular fluid soon led to its isolation from other sources as shown in table 1.

QUANTITATIVE TEST OF THE HORMONE

After the isolation of the oestrous producing hormone there was need of a method of measuring, in a comparative manner, the potency of extracts of the hormone.

The *rat unit* devised by Allen and Doisy (4) is defined as follows: "The quantity of material necessary to induce oestrous

as judged by the smear method in an ovariectomized sexually mature rat weighing 140 ±20 grams." Allen and Doisy further state, "That for physiological reasons we generally make three subcutaneous injections at intervals of four hours. The rat unit then is the minimum amount so injected which produced full oestrous growth in the genital tract. Although we are convinced that this method of testing is roughly quantitative, we have not yet made a careful study of its limitations."

Although the rat unit is recognized as having certain limitations as shown by Allen and collaborators (4), Coward and Burns (8), Evans and Burr (10), and Bugbee and Simond (7) it affords a fairly accurate quantitative test of the potency of the extracts studied.

For other information concerning the ovarian hormone the reader is referred to the reviews by Allen and Doisy (5) and Parkes (23).

OBJECT OF THE INVESTIGATION

The early work clearly showed that there is a close relation between the reproductive organs and the mammary gland. The exact cause of the growth of the mammary glands, however, has been a subject of great speculation. All the parts of the gonads, the placenta, and the fetus have been suggested as the source of the hormone which stimulates the growth of the mammary gland during pregnancy. Upon the isolation of the oestrous producing hormone and the development of the rat unit test the question immediately arose as to the effect of this hormone on the growth of the mammary gland.

There are two methods of attack of the problem. One method consists in injecting definite amounts of the hormone into immature gonadectomized experimental animals and observe the extent of the growth of the mammary gland and the initiation of milk secretion. It is interesting to note that experiments of this type are beginning to be reported which would indicate that the oestrous producing hormone is capable of producing some growth of the gland. In preliminary work, we have been able to

induce considerable growth of the gland in castrated male rabbits. A more extensive report of this work will be made later.

A second method of study of the influence of the oestrous producing hormone on the normal development of the mammary gland and the initiation of milk secretion consists in correlating the amount of the ovarian hormone present in the blood (Fels (11)) during various stages of pregnancy with the growth of the gland and the yield of a secretion.

Preliminary studies with blood were unsatisfactory due to the amount of blood required. Upon the discovery of Veler and Doisy (25) that the hormone in the blood of pregnant women was being excreted in the urine, a simple method was available by which to study the changes in the concentration of the hormone in the blood and which is excreted in the urine during pregnancy when the growth of the gland is most rapid. That the hormone is also excreted in the urine of pregnant cows was immediately demonstrated in a few preliminary tests. Recently Hisaw and Myers (17) report the presence of the hormone in the urine of a limited number of pregnant cows.

The object of the present paper is to report the results of an extensive series of tests of urine of cows in all stages of pregnancy and to show that the increase in the concentration of the hormone parallels the growth of the mammary gland of first calf heifers.

PROCEDURE

The urine for this investigation was collected from cows of the Holstein, Jersey, Guernsey, and Ayrshire breeds of the Missouri Station dairy herd. At the beginning it was necessary to watch the cows continuously to obtain samples of urine for a definite period. Later a method was discovered whereby urination was affected so that collections were made at two hour intervals.

The method consisted of a gentle massage, starting below the ventral commissure of the vulva and taking its course upward and laterally terminating beside the labium vulva (see fig. 1). After a short period of stimulation urination was produced.

While there have been published complicated methods of extracting the ovarian hormone from urine, Doisy (unpublished

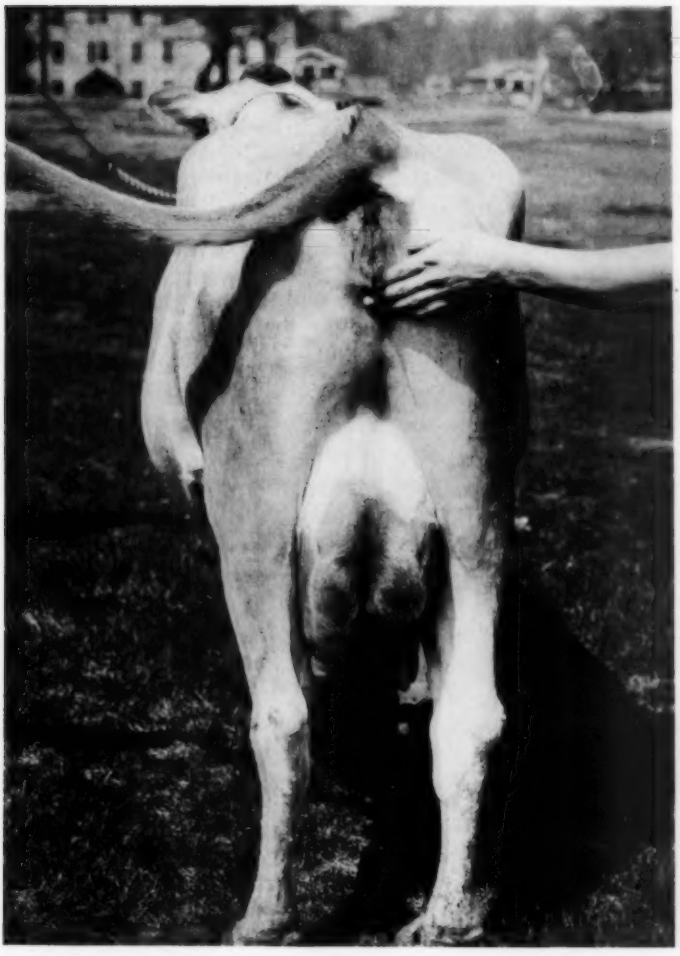

FIG. 1. THE POSITION OF THE HAND BELOW THE VENTRAL COMMISSURE OF THE VULVA, WHERE IT IS PLACED AT THE START OF THE MASSAGE WHICH CAUSES THE COW TO URINATE

From this position the hand takes its course upward and laterally terminating beside the labium vulva.

work) has devised an extremely simple procedure which was followed in all of our work. It was found that oil (olive oil was used) has an affinity for the hormone when mixed with the urine.

The method employed was to add 50 cubic centimeters of oil to 2 liters of urine or a proportional amount of oil to larger or smaller volumes of urine. After the addition of the oil, the sample was mixed for one hour and then the mixture was placed in a separatory funnel until the urine and oil had formed separate layers. The urine was then drained off and discarded while the oily residue was drained into test tubes. The oily residue was cleared in a centrifuge, pipetted off, and placed in a clean stoppered test tube for a later quantitative determination.

Allen and Doisy rat unit test was used in determining the potency of the extracts. At the time of the first injection a vaginal smear was obtained as a check to definitely show that the animal was in the dioestrous stage. Vaginal smears were obtained from the injected rats forty-eight and fifty-six hours after the time of injection. These slides were then fixed by means of a flame and stained in Delafield's hematoxylin and eosin.

Slides upon microscopic examination showing cornified cells were designated as positive. The minimum amount of the hormone to produce this condition was considered one rat unit. Slides showing a mixture of leucocytes and epithelial cells were designated as a plus minus reaction. This type of smear suggests a small amount of development but not enough to be positive. The predominance of leucocytes and to a less extent nucleated epithelial cells were designated as a minus.

PRESENTATION OF DATA

Because of the lack of information as to the efficiency of the method of extraction, considerable preliminary work was necessary. It was found that when 10, 15, or 20 cc. of oil were added to two liters of urine practically the same number of rat units were extracted. Similarly, the length of time of stirring apparently had little effect on the efficiency of the absorption of the hormone by the oil for a stirring period of thirty minutes gave results identical with the 120-minute period.

The efficiency of the method of extracting the hormone was further tested by adding known amounts of the hormone to urine and then extracting and testing each sample. It was found that the number of rat units extracted was practically identical

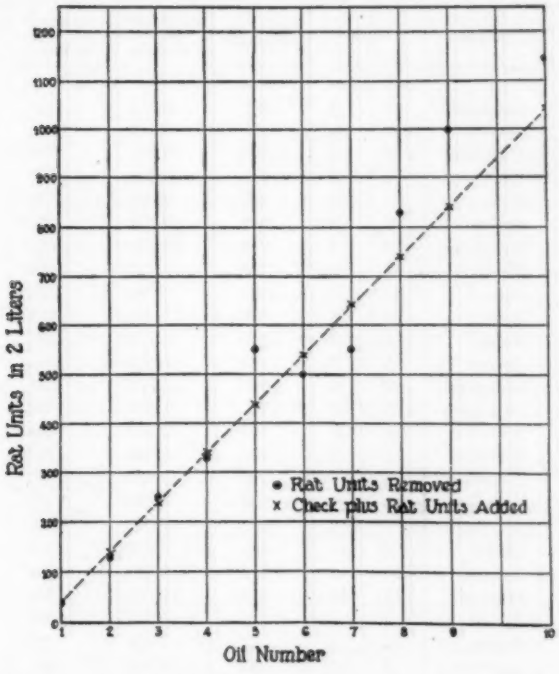

FIG. 2. THE EFFICIENCY OF THE METHOD OF EXTRACTION USED WITH INCREASING AMOUNTS OF THE OVARIAN HORMONE

The broken line shows the amount of the ovarian hormone in the urine plus the number of rat units added; the dots indicate the number of rat units recovered. These results indicate that the ovarian hormone in varying concentrations is removed by oil with equal efficiency.

with the number added to the urine. The results are shown in figure 2. These data indicate further that the oil is equally efficient in removing the hormone whether in small or large concentration.

The length of the period of urine collection necessary to obtain a representative sample of the excretion of the hormone was determined by comparing the excretion by twelve-hour and twenty-four-hour periods.

TABLE 2

Rat units in cows urine at various stages of gestation

COW NUMBER	BREED	AGE		STAGE OF GESTATION	AVERAGE URINE, 24 HOURS	AVERAGE RAT UNITS, 24 HOURS	AVERAGE RAT UNITS PER LITER
		yrs.	*mos.*	*days*	*cc.*		
426	Guernsey	3	4	281	6,235	818	131
190	Jersey	3	6	281	6,605	629	95
557	Holstein	3	5	273	8,154	782	95
426	Guernsey	3	4	271	5,504	799	145
193	Jersey	3	4	270	9,450	872	92
190	Jersey	3	6	268	9,400	462	48
557	Holstein	3	5	260	10,450	480	46
193	Jersey	3	5	255	9,295	462	49
191	Jersey	3	5	254	8,184	733	89
195	Jersey	3	4	250	11,180	1,055	94
193	Jersey	3	4	248	9,078	565	62
193	Jersey	3	5	225	7,880	485	61
556	Holstein	3	5	215	10,405	463	44
569	Holstein	2	0	201	7,370	489	66
326	Ayrshire	12	1	190	9,415	364	38
569	Holstein	2	0	186	6,730	353	32
569	Holstein	2	0	179	6,826	390	57
326	Ayrshire	12	0	169	7,368	268	35
339	Ayrshire	4	3	162	7,470	442	59
156	Jersey	7	11	161	9,335	411	44
125	Jersey	12	3	159	11,590	656	56
275	Holstein	11	10	153	11,050	400	36
197	Jersey	3	0	135	7,587	308	40
340	Ayrshire	3	8	135	6,200	229	36
341	Ayrshire	3	5	133	12,890	495	38
154	Jersey	8	9	118	7,850	81	10
177	Jersey	5	7	114	7,430	195	26
520	Holstein	6	10	107	13,860	45	3
154	Jersey	8	7	99	12,310	76	6
128	Jersey	12	0	98	6,520	12	2
151	Jersey	9	1	73	12,750	51	4
266	Holstein	12	10	57	7,095	58	8
509	Holstein	8	4	34	8,842	81	9
521	Holstein	7	0	Open	10,118	0	0

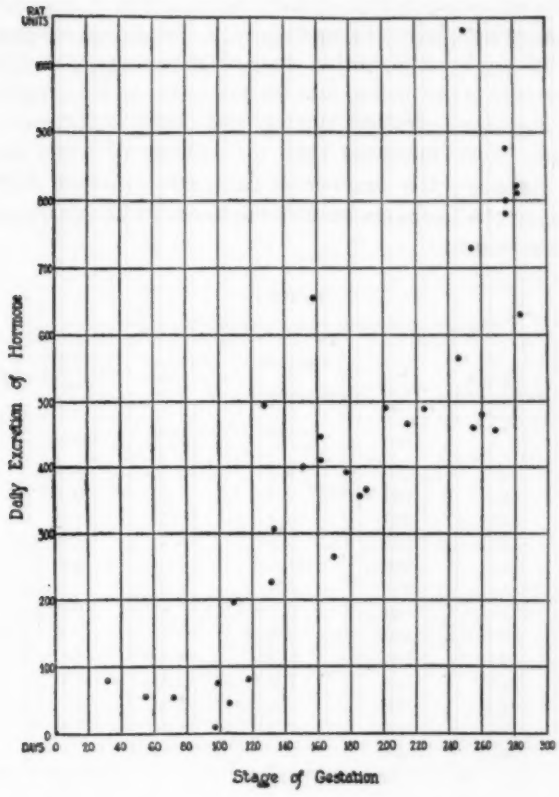

FIG. 3. THE VARIATION IN THE AVERAGE DAILY EXCRETION OF THE OVARIAN HORMONE AS EXTRACTED FROM COW'S URINE IN ALL STAGES OF GESTATION

It is seen that non-pregnant cows not in oestrum do not excrete the ovarian hormone; however, following conception there was a slight excretion of the ovarian hormone during the first 100 days. From 100 days until parturition there was a considerable increase in the daily rate of excretion amounting to 3.5 to 4 rat units daily.

The average of 12 cows from which urine was collected for forty-eight-hours indicate that although the cows show a large variation in many instances from day to day the average variation

of all the cows is negligible. As to a comparison of day and night collections (8 a.m. to 8 p.m. and 8 p.m. to 8 a.m.) an average of 26 cows in all stages of gestation showed that there were 1117 cc., or 13 per cent more urine, and 29 rat units or 6 per cent more ovarian hormone excreted during the night collections. This preliminary work indicates that the volume of urine does not seem to influence the number of rat units excreted daily. In other words, the hormone excreted appears to be independent of the volume of urine.

TABLE 3

Results of alcoholic fractionation of oil extracts

COW NUMBER	AVERAGE NUMBER OF COLLECTIONS	STAGE GESTATION	POTENCY OF OIL EXTRACTS	POTENCY OF ALCOHOLIC EXTRACTS	ALCOHOLIC FRACTIONATION INCREASE	PERCENTAGE INCREASE
		days	*rat units per cc.*	*rat units per cc.*	*rat units*	
193	3	270	8.7	18.6	9.9	113
190	2	268	4.4	8.9	4.5	102
557	3	260	4.8	11.6	6.8	140
193	2	255	5.5	18.3	12.8	233
195	1	250	11.1	16.6	5.5	49
569	5	201	3.9	7.6	3.7	98
326	5	169	3.1	3.7	0.6	22
156	2	161	4.1	8.0	3.9	115
340	2	135	2.3	8.3	6.0	266
154	1	99	1.0	2.0	1.0	100
266	1	57	0.6	2.1	1.5	250
509	1	34	0.7	2.5	1.8	257
521	4	Open	0	0		

This preliminary work indicates that a twenty-four-hour sample of urine from which the ovarian hormone has been extracted with oil is a satisfactory method for measuring the ovarian hormone content of urine with the advancing stage of pregnancy. Therefore, using this method urine from cows in various stages of gestation was analyzed for its ovarian hormone content. Table 2 shows the results. It will be noted that there were included in the group of cows non-pregnant animals and others in all stages of gestation up to 281 days. Beginning with cows not in oestrum, it was found that no ovarian hormone was excreted.

During the first 100 days of pregnancy only a slight amount of the hormone is excreted. After approximately 100 days the daily excretion begins to increase. As shown in figure 3 there is con-

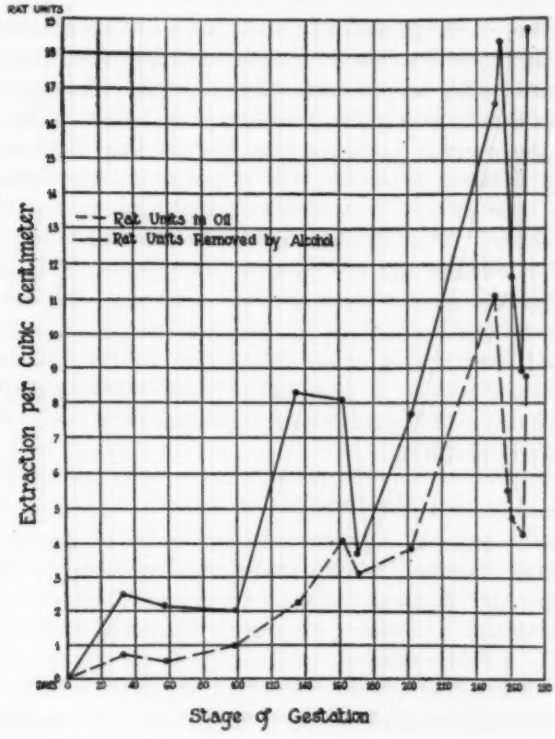

FIG. 4. RAT UNITS REMOVED BY ALCOHOLIC FRACTIONATION OF THE OIL CONTAINING THE OVARIAN HORMONE

Oils obtained from urine of cows at all stages of gestation were used for the alcoholic fractionation. The broken line shows the ovarian hormone present in the oil before extraction, the solid line after extraction with alcohol.

siderable variation in the increase for individual animals; however, the average rate of increase is approximately 3.5 to 4 rat units daily up until parturition.

In addition to the presence of the oestrous producing hormone in the urine of pregnant cows, our data indicate the possible presence of a second factor which may inhibit the usual reaction of the ovarian hormone.

A number of samples of oil containing a known amount of the ovarian hormone obtained by the previous described method were fractionated with 95 per cent ethyl alcohol. The method employed in this fractionation process was as follows: The oil containing the ovarian hormone was well shaken with about ten times its volume of alcohol, the mixture was then allowed to separate, the alcohol was then removed and evaporated to a small volume. The residue was then taken up with a small amount of oil and the evaporation completed. This oil when retested gave a greater number of rat units per cubic centimeter than was in the original oil as is shown in table 3. It will be noted that there is an increase due to alcoholic fractionation from 22 to 266 per cent or an average increase of practically 125 per cent. Figure 4 shows the average increase for cows in the various stages of gestation.

DISCUSSION OF RESULTS

From the time of puberty there are obvious changes taking place in the mammary glands of the female, whereas in the male no further development ordinarily occurs. The source of the stimulation which produces such profound changes in the female mammary gland especially during pregnancy has long been sought. The earlier theories referred the source of the stimuli to reflexes originating in the nervous system. The experimental work of that period was chiefly concerned with the possibilities of normal growth of the mammary gland taking place if severed from the usual nervous connections. In recent years with the development of the hormone theory, the work has shifted to a study of the source of hormones which may activate the mammary glands.

Lane-Claypon and Starling (19) were the first to apply experimental methods to the study of the problem. From their work with extracts of the fetus and the placenta, the theory was

advanced that the fetus was the source of the hormone which stimulates the growth of the mammary gland during pregnancy. Later others suggested the corpus luteum hormone and the so-called follicular or ovarian hormone as the source of the stimulus.

With the isolation and the perfection of a test of the ovarian or oestrous producing hormone, it became possible for the first time to experimentally determine the part played by the hormone in stimulating the growth of the mammary gland and the initiation of milk secretion.

As already suggested there are two modes of approach to the problem. It is possible to inject the purified hormone into experimental animals and note the effect produced in the gland or to determine under normal conditions of pregnancy the possible presence of the hormone in the circulation and in the urine.

From the anatomical studies of Hammond (16) who observed the most pronounced development of the mammary glands in first calf heifers during the latter months of gestation it might be expected that the greatest concentration of the hormone would be found at the same time. The data presented above are in accord with these observations and is believed to definitely prove for the first time that the oestrous producing hormone is actually present in the blood during pregnancy in increasing amounts which might be sufficient to produce the changes observed.

If the oestrous producing hormone is in part at least responsible for the growth of the mammary gland, it would be expected that the development of a large mammary gland would require a greater effective concentration of hormone than would the development of a small gland. Stated in other words, one cause of variation in the size and degree of development of the mammary glands may be due to the variation in the effective concentration of the hormone in various animals during pregnancy. Here again the data are in agreement with expectation for considerable variation in the excretion of the hormone at the same stage of gestation was observed. However, further observations will be necessary before the variations can be definitely proven to be due to genetic differences.

In addition to the presence of the oestrous producing hormone

in the urine of pregnant cows, our data indicate the possible presence of a second factor which may inhibit the usual reaction of the ovarian hormone.

In explanation of these results it is postulated that there is present in the urine and absorbed by the oil some agent which has an inhibitory effect on the action of the oestrous producing hormone. When the original oil is tested, the presence of this factor reduces the apparent potency or hormone concentration. Upon separation or fractionation of the hormone from the original oil, this inhibiting agent is either not removed or is removed to a less extent than is the ovarian hormone.

It is well known that the vaginal smears of pregnant animals indicate an anoestrous condition. This phenomena is difficult to explain unless it is assumed that some inhibitory agent or hormone is present preventing the oestrous condition even when large amounts of the oestrous producing hormone are being excreted by such animals.

SUMMARY

1. A simple efficient method of extracting the ovarian hormone from the urine of pregnant cows by the use of oil is described.

2. As a result of the study of the diurnal variation in the volume of urine and its ovarian hormone content on a twelve-hour basis, it was found that the excretion of urine from 8 p.m. to 8 a.m. exceeded the collection during the day by 1117 cc. or 13 per cent; however, the difference in the excretion of the ovarian hormone was insignificant. For successive twenty-four-hour collections, little variation either in volume of urine or number of rat units was observed.

3. The yield of ovarian hormone is generally independent of the volume of urine excreted during a twenty-four-hour period.

4. Beginning with non-pregnant cows which excrete the ovarian hormone only at oestrum it was shown that following conception there was a slight excretion of the ovarian hormone during the first one hundred days. From one hundred days until parturition there was a considerable increase in the rate of excretion of the ovarian hormone, amounting to 3.5 to 4 rat units per day.

5. Following the alcoholic extraction of oils containing the ovarian hormone, it was found that there was an apparent increase in the number of rat units present in a definite volume of oil. These results are taken to indicate that there is present in the urine, and absorbed by the oil, some agent which has an inhibitory effect on the ovarian hormone. A tentative theory is advanced that this hormone or factor is responsible for maintaining the anoestrous type of cells in the vagina of pregnant animals.

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PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The annual meeting of the American Dairy Science Association was held in the new National Museum Building, Washington, D. C., on June 26 to 28, 1929. It was the third summer meeting with the program as a symposium of new completed research and activities, or reports of work in progress. The program was arranged by H. P. Davis of Nebraska, H. A. Ruehe of Illinois, and C. H. Eckles of Minnesota.

Members of the Bureau of Dairy Industry under the chairmanship of L. A. Rogers arranged special tours and entertainment of educational and historic interest for the ladies. The entire Association visited the Beltsville Farm on the afternoon of June 27th to inspect the studies in dairy cattle breeding, feeding, and management conducted by the Bureau of Dairy Industry. The following afternoon tours were made to Arlington and Mount Vernon. The banquet held at the Cosmos Club on the evening of the 27th, with H. A. Ruehe as toastmaster, was followed by short addresses by President G. C. White of Connecticut, by L. A. Rogers of Washington, D. C., and by F. B. Morrison of New York who gave an interesting illustrated address on his observations in Germany as a member of the Agricultural commission.

The executive committee meeting, attended by White (presiding), Ruehe, Sherman, Dahlberg, and Allen, considered several items of general interest. It was believed that the meeting place should be decided and announced a year in advance so that members can plan their work and vacations with a knowledge of the time of the next convention. The next meeting place will be Ames, Iowa and the committee tentatively accepted the invitation of the Pacific Coast members to hold the 1931 meeting in California, subject to later consideration for final decision. The committee decided that some effort should be made to further

extend the field of the Journal and requested Dahlberg to plan for such extension.

President White appointed the following committee on nominations:

A. A. Boland (chairman)
C. H. Eckles
H. B. Ellenberger
H. W. Gregory
C. L. Roadhouse

This committee gave the following report:

President	J. M. Sherman H. P. Davis
Vice-President	H. C. Jackson C. R. Gearhart
Secretary-Treasurer	Fordyce Ely R. R. Graves
Editor	A. C. Dahlberg

The various sections which elected officers chose the following men:

Dairy Production	H. B. Ellenberger, chairman C. W. Turner, secretary
Dairy Manufacturers	C. L. Roadhouse, chairman C. D. Dahle, secretary

The committee on resolutions gave several resolutions which were quickly adopted and one, which after lengthy discussion, was tabled. Mention should be made of reports on the committees on chemical and on bacteriological analysis of milk and its products. The following resolutions of the committee on score cards was adopted:

We, your committee on Legal Standards and Score cards, recommend that the score card be used as a guide in milk scoring contests grade milk as perfect when it complies in composition with the requirements of the state in which the contest is held.

The following program was presented.

GENERAL SESSION

June 26

President G. C. White, presiding

Address of Welcome. A. F. Woods, Director of Research, U. S. Department of Agriculture.

Address of the President. G. C. White, Connecticut Agricultural College.

Announcements.

The Bureau of Dairy Industry. O. E. Reed, Bureau of Dairy Industry, U. S. Department of Agriculture.

Economic Aspects of Diseases of Dairy Cattle. J. R. Mohler, Bureau of Animal Industry, U. S. Department of Agriculture.

Present Trends of Business and Economic Development of the Dairy Industry. Roy C. Potts, Bureau of Agricultural Economics, U. S. Department of Agriculture.

Some Phases of the Phosphorus Deficiency Problem in the Nutrition of Dairy Cattle. C. H. Eckles, University of Minnesota.

Effect of Feeding Flourine Upon the Health and Teeth of Dairy Cattle. George E. Taylor, Michigan State College.

The Nervous Reaction of Animals on a Deficiency Diet (Film). J. S. Hughes and H. W. Cave, Kansas State Agricultural College.

Discussion.

Buffet Lunch in Laboratories of Bureau of Dairy Industry.

Nutritional Value of Raw and Pasteurized Milks. J. M. Brannon and M. J. Prucha, University of Illinois.

The Taste of Market Milk as Influenced by Certain of its Constituents. C. L. Roadhouse, University of California.

Metals and Milk. E. S. Guthrie, Cornell University, and C. L. Roadhouse, University of California.

Buffer Action of Milk:

a. Factors that Determine the Buffer Action of Milk. E. O. Whittier, Bureau of Dairy Industry, U. S. Department of Agriculture.

b. Variations in the Buffer Value of Herd Milk. Paul D. Watson, Bureau of Dairy Industry, U. S. Department of Agriculture.

c. Buffer Value of Proprietary Milks and Milks from Different Individuals. George E. Holm, Bureau of Dairy Industry, U. S. Department of Agriculture.

Value of Photography in Dairy Cattle Breeding and Type Investigations. M. H. Fohrman, Bureau of Dairy Industry, U. S. Department of Agriculture.

Excretion of Ovarian Hormones during Pregnancy. C. W. Turner and C. W. Nibler, University of Missouri.

Philadelphia Price Plan. I. Ralph Zollers, Inter-State Milk Producers Association.

Relationships of Temperature to Physical, Chemical and Biological Changes in Milk. J. C. Marquardt, New York Agricultural Experiment Station.

College Creameries. H. A. Ruehe, University of Illinois.
Discussion.
Meeting for Organization of Dairy Economic Section.
Receptions to Members. Hotel Harrington.

Dairy Manufacturers—Bacteriology—Market Milk

June 27

W. H. Martin, presiding

Estimation of Fat and Phospholipins in Buttermilk. L. M. Thurston, University of West Virginia.
Fat Determination in the Evaporated Milk Industry. Frank S. Rice, Evaporated Milk Association.
Some Chemical Changes in Frozen Milk Occurring in Storage. E. O. Anderson, Connecticut Agricultural College.
Effect of Heat Treatment on the Stability or Viscosity of Cultured Buttermilk. R. W. Bell, Bureau of Dairy Industry, U. S. Department of Agriculture.
A Study of Washed Curd Cheese. W. V. Price and Walter Hochstrasser, University of Wisconsin.
Relation of Cheese Composition to Quality. J. L. Sammis and Leo Germain, University of Wisconsin.
Some Observations on Process Cheese. H. L. Templeton and H. H. Sommer, University of Wisconsin.
Rennet Coagulation of Milk from Individual Cows. F. L. Schacht and H. H. Sommer, University of Wisconsin.
A Formula Expressing the Relation between Fat and Protein in Milk. A. E. Perkins, Ohio Agricultural Experiment Station.
Comparison of Methylene Blue, Janus Green and Plate Counts in Grading Raw Milk. M. J. Prucha, University of Illinois.
The Foaming of Milk. C. S. Leete, Bureau of Dairy Industry, U. S. Department of Agriculture.
Some New Facts Concerning Ropy Milk. C. N. Stark and Pauline Stark, Cornell University.
The Bacterial Proteolytic Enzymes. Their Activity of Different pH values and the Effect of Sodium Chloride. E. H. Parfitt and George Spitzer, Purdue University.
Committee Reports and Election of Officers of Manufacturing Section.
Inspection of Beltsville Farm and Experimental Work in Dairy Cattle Breeding, Nutrition and Management.
Banquet—Cosmos Club.

Official Testing and Dairy Production

June 27

J. R. Dice, presiding

A Statistical Study of the Reliability of Bi-Monthly Tests. Warren Gifford, University of Missouri.

Advantages of Bi-Monthly Testing. Lynn Copeland, American Jersey Cattle Club.

Studies of the Bi-Monthly Test. J. C. McDowell, Bureau of Dairy Industry, U. S. Deparment of Agiculture.

Discussion.

R. B. Becker, presiding

The Influence of Low Fat Ration on Yield of Milk and Fat. L. A. Maynard and C. M. McCay, Cornell University.

Various Methods of Comparing Transmitting Ability of Dairy Sires by Daughter and Dam Comparison. C. W. Turner, University of Missouri.

Roughage Grinding. Thomas Olson, South Dakota State College

Digestibility of the Total Ration as Affected by Ground Versus Unground Roughage. Paul S. Williams, Pennsylvania State College.

Pasture Fertilization. C. H. Parson, Synthetic Nitrogen Products Corporation.

Yield and Composition of Pasture Grass. H. B. Ellenberger, University of Vermont.

Significance and Causes of Daily Fluctuation in Weight in Dairy Cattle. C. L. Allen and R. G. Maxwell, Cornell University.

Wintering Dairy Heifers by Means of the Self Feeder. H. O. Henderson, University of West Virginia.

Committee Reports and Election of Officers of Production Section.

Inpection of Beltsville Farm and Experimental Work in Dairy Cattle Breeding, Nutrition and Management.

Banquet—Cosmos Club.

DAIRY EXTENSION

June 28

G. A. Williams, presiding

The Relation of the Bureau of Dairy Industry to the Office of Coöperative Extension Work. C. B. Smith, Office of Coöperative Extension Work, U. S. Department of Agriculture.

Supervision of Dairy Herd Improvement Associations. C. R. Gearhart, Pennsylvania State College.

Future Development of Dairy Herd Improvement Association. A. J. Cramer, University of Wisconsin.

District Conference for Cow Testers. Floyd Johnson, Iowa State College.

Coöperative Creameries as a Means for Promoting Herd Testing. H. R. Searles, University of Minnesota.

Scoring Type and Pedigree as a Stimulus to Proving the Herd Sire. E. J. Perry, New Jersey College of Agriculture.

Scoring Dairy Sires and Their Progeny. M. J. Regan, University of Missouri.

Bull Associations in Pennsylvania. S. J. Brownell, Pennsylvania State College.

Development and Promotion of 4 H Calf Club Activities. R. A. Turner, Office of Coöperative Extension Work, U. S. Department of Agriculture.

Timely Dairy Information and Subject Matter for Dairy Extension. A. C. Baltzer, Michigan State College.

Some Results of Recent Tabulations of Dairy Herd Improvement Association Records. J. C. McDowell, Bureau of Dairy Industry, U. S. Department of Agriculture.

Use of Local Leaders in Work of Dairy Herd Improvement Association. J. H. Brock, University of Illinois.

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June 28

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Keeping Quality and Shrinkage of Print Butter in Storage. William White, Bureau of Dairy Industry, U. S. Department of Agriculture.

Quantitative Changes in the Microflora of Butter. H. Macy, University of Minnesota.

Sandiness in Nut Ice Cream. W. H. E. Reid, University of Missouri.

Use of Honey in Ice Cream Manufacture. P. H. Tracy, University of Illinois.

Utilization of Fruits in Frozen Dairy Product. H. J. Mack, Massachusetts State College.

Relationship of the Amount and Quality of Gelatin to the Viscosity and Plasticity of the Ice Cream Mix. Alan Leighton and Floyd E. Kurtz, Bureau of Dairy Industry, U. S. Department of Agriculture.

Study of the Effect of Aging Ice Cream Mixes. J. C. Hening, New York Agriculture Experiment Station.

Effect of Dried Skimmilk on the Texture and Flavor of Ice Cream. O. E. Williams and S. E. Hall, Bureau of Dairy Industry, U. S. Department of Agriculture.

Effect of Homogenization on Protein Stability. E. J. Doan, Pennsylvania State College.

Factors that Control the Color of Evaporated Milk. B. V. Webb and George E. Holm, Bureau of Dairy Industry, U. S. Department of Agriculture.

Evaporated Milk and Its Place in the Diet. Frank S. Rice, Evaporated Milk Association.

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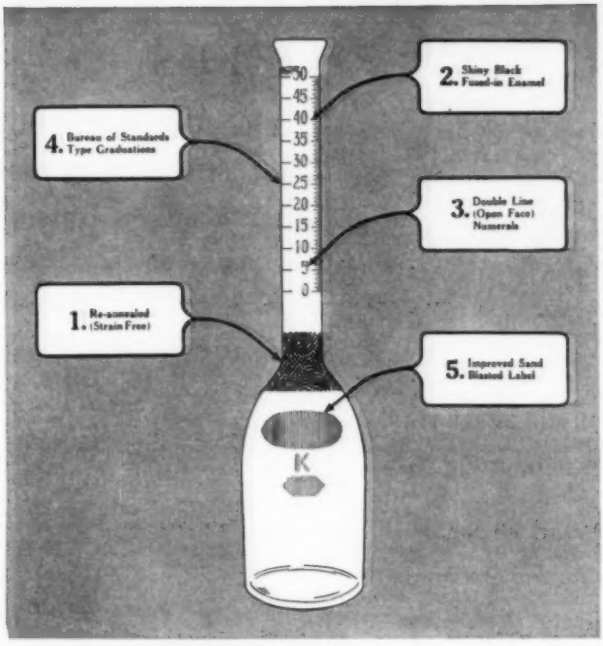

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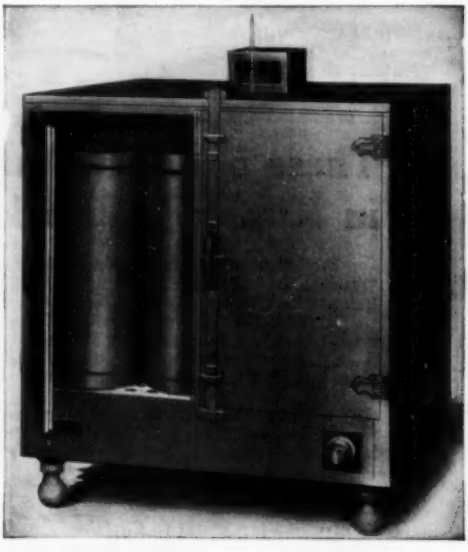

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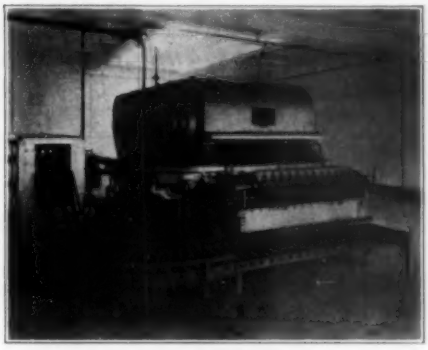

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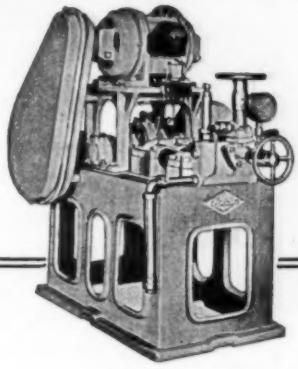

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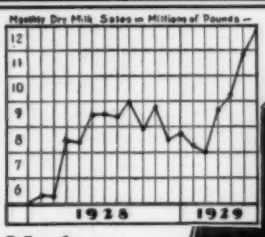

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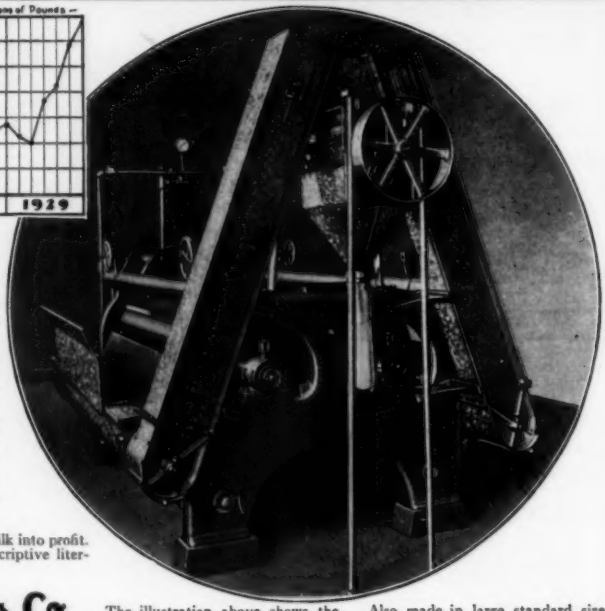

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